

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



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Señor

SUPERINTENDENTE DE INDUSTRIA Y COMERCIO

DIVISIÓN DE NUEVAS CREACIONES

E. S. D.

Re: PI.-**ABBVIE, INC.**-Solicitud de Patente de Invención en Fase Nacional PCT para "**FORMULACION DE LIPIDOS ESTABILIZADA DE UN PROMOTOR DE LA APOPTOSIS**".-Clase **A61K 9/0**. -Expediente número **11-162.226**.

1. Me refiero al auto dictado por ese Despacho notificado por fijación en lista de 30 de Abril de 2013.

2. Con el fin de responder las objeciones expuestas por el Señor Examinador en su Concepto Técnico correspondiente al Oficio No. **7621**, en relación con la Claridad del Capítulo Reivindicatorio y el Nivel Inventivo de la presente solicitud, me permito dirigir su atención a las siguientes modificaciones y argumentos técnicos por medio de los cuales no sólo se superan completamente dichas objeciones sino que además se establece de manera incontrovertible la patentabilidad de la solicitud en estudio.

3. **EXCEPCION A LA PATENTABILIDAD.**

De otro lado, respecto a las reivindicaciones **32** a **40** actualmente presentadas el Señor Examinador señala que se relacionan con un método de tratamiento lo cual no es materia considerada como patentable.

En este sentido me permito resaltar la materia considerada en las reivindicaciones **32** a **40** han sido **eliminadas**.

Por lo tanto, el mencionado nuevo capítulo reivindicatorio no se refiere de ninguna manera a usos y se enmarca así dentro de lo establecido en el Artículo 20 literal d de la Decisión Andina 486 de 2000.

4. **CAPÍTULO REIVINDICATORIO.**

En primer lugar, el Señor Examinador señala que la reivindicación 1 carece de concisión dado que no limita el alcance de la invención por el empleo de términos ambiguos y generales.

Así mismo, el Señor Examinador afirma que el capítulo reivindicatorio incluye materia que no encuentra sustento en la memoria descriptiva por el empleo de términos como prodrogas, sales de prodrogas o un metabolito.

Tomando en consideración estas objeciones me permito manifestar que es deseo del solicitante presentar un **nuevo capítulo reivindicatorio**, que remplace al actualmente presentado, el cual se refiere de forma clara y concisa a realizaciones preferidas de la invención en concordancia con las revelaciones de la descripción de la solicitud.

En conexión con el mencionado nuevo capítulo reivindicatorio me permito resaltar que este no se constituye de ninguna manera en una ampliación al alcance de la invención, tal como fue originalmente presentada, toda vez que dicho capítulo define de forma más precisa la composición farmacéutica que es objeto de la presente invención de acuerdo con las enseñanzas de la descripción, tal como se expone a continuación:

En respuesta a la objeción de la reivindicación 1 respecto a los términos "antioxidante calcógeno" y "más pesado", el Solicitante señala que estos términos no deberían considerarse por separado ya que el término correcto es "antioxidante calcógeno más pesado" y en la memoria descriptiva se provee una definición para este término completo. La frase correcta y completa "antioxidante calcógeno más pesado" se define claramente en el párrafo [0035] de la memoria descriptiva.

Un "antioxidante calcógeno más pesado" o "HCA" es un compuesto con propiedades antioxidantes que contiene uno o más átomos de azufre o selenio oxidable, más en particular de azufre.

De acuerdo a esta clara definición, el término reivindicado "antioxidante calcógeno más pesado" se refiere a un compuesto que comprende un selenio o un azufre "oxidable" y que tiene propiedades antioxidantes. El Solicitante provee una estructura general de los HCA en el párrafo [0098] de la memoria descriptiva y enumera múltiples ejemplos de tales compuestos en el párrafo [0106] de la memoria descriptiva. En

consecuencia, el término "antioxidante calcógeno más pesado" es completamente claro en el contexto de las reivindicaciones.

Además, la reivindicación 1 se modificó para proveer una estructura claramente definida para el "antioxidante calcógeno más pesado farmacéuticamente aceptable". Ahora, las reivindicaciones independientes 10 y 30, también definen clases claras y limitadas de antioxidantes calcógenos más pesados. Los antioxidantes de la reivindicación 10 tienen sustento en el párrafo [0106] de la memoria descriptiva. Los antioxidantes de la reivindicación 30 tienen sustento en el párrafo [0113] de la memoria descriptiva.

El término "vehículo lípido" es también claro en el contexto de las reivindicaciones que definen una composición farmacéutica. Es conocido en el arte que los componentes activos se formulan con uno o más vehículos (también llamados excipientes). El párrafo [0109] de la memoria descriptiva divulga que el sistema de vehículo puede comprender un solo material lipídico o una mezcla de dos o más de tales materiales. La mayor parte de la memoria descriptiva describe materiales lipídicos, en particular, materiales fosfolipídicos para usarlos en el "vehículo lípido".

Por último, el término "sustancialmente no acuoso" es también claro en el contexto de las reivindicaciones y de la memoria descriptiva. En el párrafo [0119] se especifica que esta cantidad sea menor que un 5% en peso de agua. Se han modificado las reivindicaciones de acuerdo con esta definición.

En conexión con lo anterior el nuevo capítulo reivindicatorio ha sido modificado de la siguiente manera:

- **Reivindicación 1 y 7 (Nueva reivindicación 1).** Se modificaron las reivindicaciones mencionadas de manera que se eliminó el término "prodroga, la sal de una prodroga o metabolito de la misma". Además, se modificó el pliego de reivindicaciones de manera de evitar el uso del término "aproximadamente". La materia reclamada en la reivindicación 10 actualmente presentada, se incluyó en la nueva reivindicación 1.

- **Reivindicaciones 2 a 9 (nuevas reivindicaciones 2 a 9).** Corresponden a las actualmente presentadas.

- **Reivindicación 10 (Nueva reivindicaron 10).** Corresponde a una reivindicación independiente y se incluyo la materia reclama en la reivindicación 11, que tiene sustento en el párrafo [0106].
- **Reivindicación 11.** la materia reclamada en la reivindicación en mención se incluyo en la nueva reivindicación 1.
- **Reivindicaciones 12 a 23 (Nueva reivindicación 11 a 22).** Se modifiko la dependencia y numeración, corresponde a la materia reclamada en la reivindicaciones 12 a 23 actualmente presentadas.
- **Reivindicaciones 24.** La materia reclamada en la reivindicación 24 actualmente presentada, se incluyo en la nueva reivindicación 30 independiente, que tiene sustento en el párrafo [0113] de la memoria descriptiva.
- **Reivindicaciones 25 a 31 (Nuevas reivindicaciones 31 a 37).** Corresponden a las actualmente presentadas.
- **Reivindicaciones 32 a 40.** Estas reivindicaciones han sido **eliminadas** del capítulo reivindicatorio.

En vista de las modificaciones realizadas a las reivindicaciones y lo divulgado en la memoria descriptiva, deberían retirarse todos los rechazos por falta de claridad, concisión y sustento.

De esta forma, el capítulo reivindicatorio modificado está constituido por **37 reivindicaciones** que se refieren de manera clara y concisa a las realizaciones preferidas de la presente invención.

En conexión con todo lo discutido anteriormente, me permito señalar que el mencionado nuevo capítulo reivindicatorio supera completamente las objeciones presentadas por el Señor Examinador a este respecto y por lo tanto dicho capítulo se enmarca en su totalidad dentro de lo preceptuado en el Artículo 30 de la Decisión Andina 486 de 2000.

5. **NIVEL INVENTIVO.**

Finalmente, el Señor Examinador considera la composición farmacéutica de la presente invención carecerían de altura inventiva a la luz de las enseñanzas de los documentos: **US 2007/0027135 (D1)** y **US 5665379 (D2)**.

En conexión con lo anterior, el Señor Examinador asegura que para la persona versada en la materia sería evidente obtener la composición farmacéutica de la presente solicitud incluyendo las partículas lipídicas como vehículos, conocidas en el documento D2 en la composición divulgada en el documento D1.

Sobre este particular me permito señalar que existen diferencias significativas entre las enseñanzas de los documentos citados y la presente invención que harían que la persona versada en la materia de ninguna manera pudiera obtener la composición farmacéutica reclamada por la solicitud en estudio a partir de dichas enseñanzas del estado del arte, tal como se discutirá a continuación.

Las reivindicaciones modificadas son novedosas e inventivas respecto a las referencias D1 (Bruncko et al., U.S. 2007/0027135) y D2 (Herslof et al., U.S. 5,665,379), solas o en combinación. En consecuencia, debería retirarse el rechazo de las reivindicaciones respecto a esta combinación.

El Solicitante ha inventado y divulgado una composición farmacéutica para suministrar en forma oral capaz de alcanzar altas cargas de un compuesto inhibidor de Bcl-2 activo, tal como el ABT-263. Por ejemplo, el Solicitante divulga, en el párrafo [0088] de la memoria descriptiva, formulaciones en las cuales la solubilidad del ABT-263 es al menos 10 mg/mL, al menos 20 mg/mL, o aún al menos 25 mg/mL (tales como 30, 40, 50, 75, 100, 125, 150 o aún 200 mg/mL) y hasta 500 mg/mL de equivalentes de base libre (reivindicación 9). El Solicitante ha proporcionado además múltiples composiciones en los Ejemplos en los cuales la carga del fármaco es al menos 25 mg/mL, 50 mg/mL, o aún al menos 100 mg/mL. Ver el Ejemplo 10, Tabla 14, que muestra composiciones que comprenden 50 mg/mL y 100 mg/mL de ABT-263 con el antioxidante adicional y EDTA. Las formas de dosificación capaces de liberar una dosis prescrita de un compuesto farmacéuticamente activo, al mismo tiempo que impiden la liberación de una cantidad excesivamente alta de los excipientes que lo acompañan, son deseados en el campo por un número de razones.

Por ejemplo, el desarrollo de formas de dosificación de alta carga es deseable para aumentar el cumplimiento por parte del paciente, ya que las formas de dosificación concentradas evitan requerir la administración de múltiples cápsulas o grandes volúmenes para alcanzar la dosis prescrita. Las formas de dosificación de alta carga también son deseables para que el paciente pueda evitar consumir, en el caso de formulaciones lipídicas, una cantidad excesivamente alta de calorías para alcanzar la dosis prescrita. En vista de lo anterior, la divulgación del Solicitante, de una composición farmacéutica capaz de proveer altas dosis de ABT-263 (y otros inhibidores de Bcl-2 relacionados) es un logro significativo, patentable y relevante para el arte.

Además, es importante para el campo del desarrollo de formulaciones farmacéuticas que la composición sea estable frente a la degradación (por ejemplo por oxidación) del compuesto activo. Respecto a esto, las presentes reivindicaciones se refieren a una composición farmacéutica para administrar en forma oral que comprende un antioxidante calcógeno más pesado farmacéuticamente activo, el cual abarca antioxidantes que comprenden un selenio o un azufre oxidable. Como se divulga en el párrafo [0035] de la memoria descriptiva:

También se ha descubierto que no todos los antioxidantes son efectivos para inhibir dicha degradación oxidativa en un grado aceptable. Más en particular, se ha descubierto que la clase de antioxidantes que aquí se denominan "antioxidantes calcógenos más pesados" o "HCAs" muestra un rendimiento superior al respecto en comparación con los antioxidantes que se utilizan más ampliamente en el arte.

La presente invención se refiere por lo tanto a composiciones farmacéuticas capaces de alcanzar altas cargas de dosis de ABT-263 activo, e inhibidores de Bcl-2 similares, que además incluyen antioxidantes calcógenos más pesados, que protegen al compuesto activo de la degradación por oxidación de la molécula de tioeter. En el párrafo [0043] se puede ver una descripción de la oxidación del tioeter del ABT-263. Además, en la Tabla 14, se muestran ciertas formulaciones, en las cuales, los sulfóxidos formados por la oxidación del ABT-263 se mantuvieron tan bajos como un 0,22% p/p luego de un mes de almacenamiento. En algunos casos, no se pudieron detectar sulfóxidos en composiciones formuladas con antioxidantes calcógenos más pesados. Ver la Tabla 17 de la memoria descriptiva. Las composiciones sin antioxidante o las composiciones formuladas con un antioxidante distinto presentaron productos de degradación de más de un 0,6% p/p luego de cuatro semanas. Ver Tabla 3 de la memoria descriptiva.

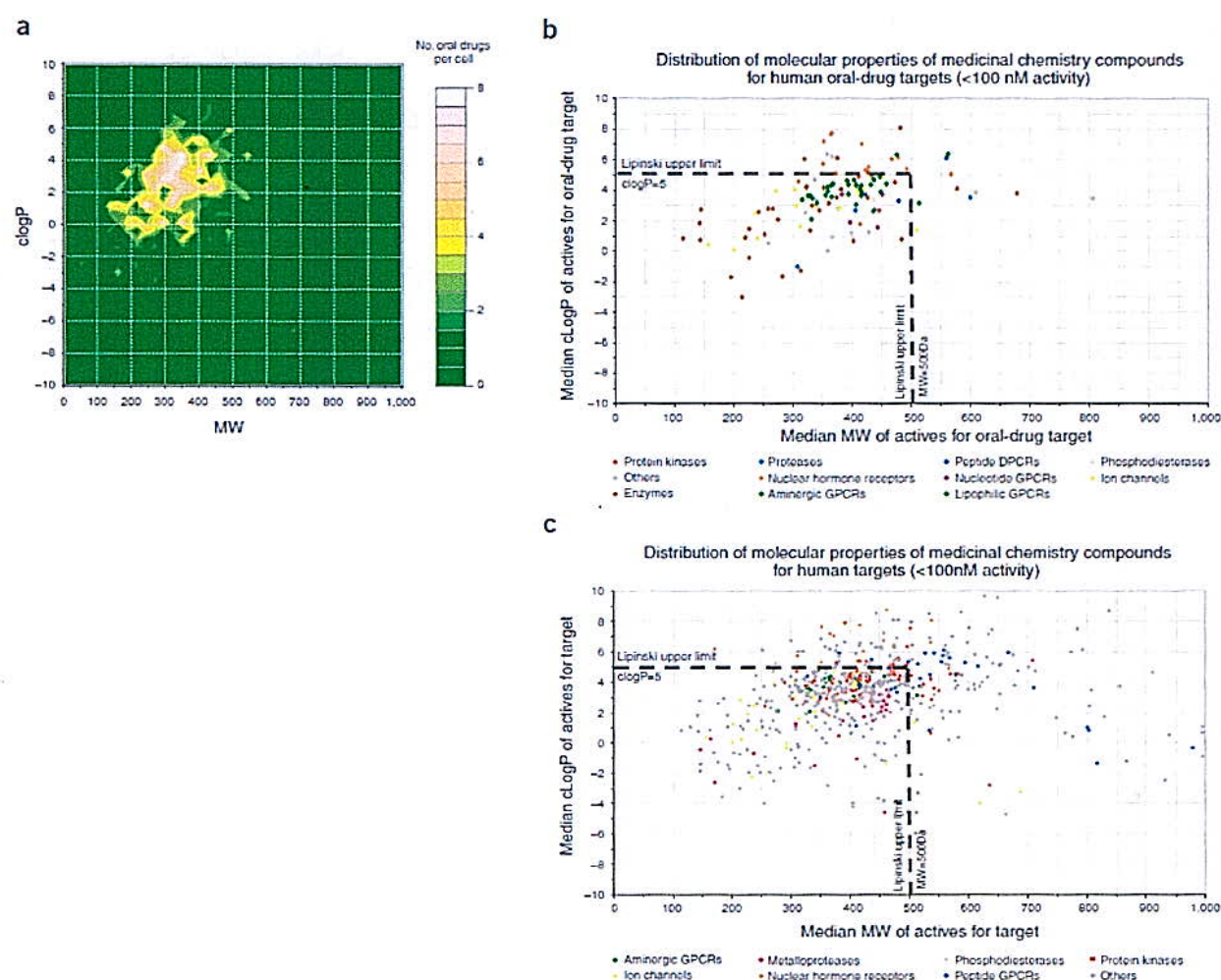
Contrariamente a la posición del Examinador, proveer una forma de dosificación adecuada para los compuestos reivindicados de Fórmula I, representados por el ABT-263, no consiste del simple hecho de combinar un vehículo, tal como el liposoma de la Referencia D2, y un antioxidante. Como se lo establece en el párrafo [0036] de la memoria descriptiva:

La preparación de una formulación en solución de ABT-263 o un compuesto de fórmula I junto con una cantidad efectiva como antioxidante de un HCA farmacéuticamente aceptable en un vehículo lípido no es simplemente cuestión de seleccionar un HCA apropiado. Se debe seleccionar un sistema de vehículo que sea capaz de mantener en solución no solo a la droga en una concentración terapéuticamente útil, sino también a una cantidad del HCA efectiva como antioxidante.

De hecho, el compuesto de Fórmula I tiene características químicas y estructurales particulares que hacen que la formulación de un vehículo adecuado sea particularmente difícil. Teniendo en cuenta la dificultad de preparar una formulación adecuada para el compuesto de fórmula I, la composición definida por las reivindicaciones tiene actividad inventiva respecto al arte previo.

La dificultad para formular una composición farmacéutica adecuada para administrar en forma oral que comprenda los compuestos de la reivindicación 1, tal como el ABT-263, se basa en parte en sus características de compuesto tipo no-fármaco, que incluyen alto peso molecular y alto valor de cLogP, el cual indica hidrofobicidad. Como se puede observar en la FIG. 6 de Paolini et al. (los gráficos que se presentan en la siguiente página fueron copiados de la Figura 6 de Paolini et al. publicado en Nature Biotechnology, vol. 24, No. 7, July 2006, pp. 805-815 y se encuentra adjunto), la mayoría de los compuestos tienen un peso molecular por debajo de 500Da y un cLogP menor que 5, y ambos cumplen con las reglas de Lipinski para el comportamiento tipo fármaco. Aunque unos pocos compuestos caen por fuera de ambos límites superiores de las reglas de Lipinski, el número de estos compuestos es significativamente menor que el número de compuestos que cumplen con las reglas. Además, pocos compuestos se desvían significativamente de los límites de las reglas de Lipinski. De hecho, como se demostrará más detalladamente más abajo, ninguno de los compuestos de administración oral descritos en los gráficos de Paolini et al. se desvían del comportamiento tipo fármaco tanto como los compuestos de fórmula I, representados por el ABT-263.

Los pesos moleculares de los compuestos que caen dentro del alcance de la Fórmula I son relativamente altos. La reivindicación 1 define los detalles estructurales significativos de los compuestos de Fórmula I, y los compuestos definidos tienen pesos moleculares relativamente altos. Por ejemplo, el ABT-263 tiene un peso molecular de 974,61 Daltons, el cual es notablemente mayor que el límite superior de Lipinski de 500 Daltons. Ver las Figuras 6b y 6c de Paolini et al. como se muestra en la Figura 6c de Paolini et al, solo algunos pocos compuestos tienen un peso molecular mayor a 900 Daltons. Como se muestra en la Figura 6b, la cual pertenece a drogas de administración oral, sólo un compuesto tiene un peso molecular que excede los 800 Daltons (y su peso molecular es menor que 900 Daltons), pero este compuesto está por debajo del límite de cLogP y por lo tanto tiene una solubilidad en agua tipo fármaco. Ninguna de las moléculas de administración oral de la Figura 6b tienen un peso molecular que exceda los 900 Daltons. El ABT-263 tiene un peso molecular de 974,61 Daltons, y por lo tanto representa un ejemplo atípico notable entre los compuestos de administración oral.



Los compuestos de las presentes reivindicaciones son además únicos, ya que su hidrofobicidad es mayor que el límite superior de Lipinski de 5. Aunque el ABT-263 es un zwitterion, y por lo tanto no tiene un valor de cLogP estándar o convencional, es posible medir un coeficiente de reparto log D entre n-octanol y agua con un pH fisiológico de 7,4. El valor de log D es aproximadamente comparable a los valores de cLogP discutidos en Paolini et al. Es de hacer notar que el ABT-263 tiene un valor de log D de 6,3, que es indicativo de una notable baja solubilidad y que es mucho mayor que el límite superior de Lipinski de 5. (Se destaca que el eje del coeficiente de reparto es el logaritmo del coeficiente de reparto, de manera que cada cambio de unidad en el valor representa un cambio de 10 veces en el coeficiente de reparto. Por lo tanto, una diferencia de una simple unidad representa una diferencia significativa de solubilidad en agua.) Nuevamente, en referencia a la Figura 6b, sólo unos pocos compuestos de los mostrados en la Tabla tienen un valor de cLogP que supera 6, pero todos los compuestos de administración oral con valores de cLogP superiores a 6 tienen pesos moleculares por debajo de aproximadamente 600. El ABT-263, tiene un valor de log D mayor que 6 por lo tanto representa un ejemplo atípico notable en este sentido, entre los compuestos de administración oral.

En vista del alto peso molecular y del alto valor de log D, los compuestos de la Fórmula I, tales como el ABT-263, están por fuera de los límites de **ambas** reglas de Lipinski para el comportamiento tipo fármaco. Además, como puede verse en los gráficos mencionados, **ningún otro compuesto activo de administración oral** presentado en los gráficos de Paolini et al. tiene las propiedades combinadas de un peso molecular de más de 900 Daltons y un log D mayor a 6. Estos valores de peso molecular e hidrofobicidad han hecho que el ABT-263 sea caracterizado como un compuesto de Clase IV de acuerdo al Sistema de Clasificación Biofarmacéutica, el cual abarca fármacos que tienen tanto baja solubilidad en agua como baja permeabilidad. Es conocido en el arte que los compuestos de clase IV son difíciles de formular en productos farmacéuticos para administración oral.

El Solicitante además hace referencia a Lessene et al. (también adjunto) en cuanto a que el ABT-263 es un compuesto de tipo no-fármaco. Lessene et al. describió al ABT-737 (peso molecular de 813,43) en la página 996:

Con respecto a la masa molecular, el número de heteroátomos, los grupos funcionales y los enlaces capaces de rotar, el ABT-737 falla sustancialmente en cumplir con las reglas clásicas de comportamiento tipo fármaco.

El ABT-263 es una molécula aún mayor, la cual también comprende un gran número de heteroátomos. En vista de lo anterior, al menos en vista de los comentarios provistos por Lessene et al., los compuestos de fórmula I, tales como el ABT-263, pueden considerarse también como de tipo no-fármaco.

En vista de lo anterior, un compuesto que caiga dentro del alcance de la fórmula I, tal como el ABT-263, es un compuesto de tipo no-fármaco y presenta el desafío de formular una composición para una administración oral adecuada. En consecuencia, la formulación de una composición para administración oral adecuada no es predecible, y una persona normalmente versada en la materia no podría haber predicho una composición adecuada recurriendo a referencias como la citada Herslof et al. El Solicitante respetuosamente señala que las presentes reivindicaciones son inventivas respecto a la combinación de referencias citadas, ya que una persona normalmente versada en la materia no podría haber predicho una composición farmacéutica adecuada para administración oral que comprenda un compuesto de fórmula I, y los componentes mencionados, en vista de las propiedades únicas del compuesto activo en términos de características de compuesto tipo no-fármaco tales como peso molecular y alta hidrofobicidad.

En particular, en relación ahora al arte previo citado, la referencia citada D1 divulga la síntesis del ABT-263 y análogos del mismo. (ver, por ejemplo, la Fórmula II de la página 1, el párrafo [0012] y la Tabla 2 de la página 12 de la publicación de Bruncko.) En sus partes relevantes, la referencia D1 divulga múltiples excipientes en el párrafo [0100], que incluyen triglicéridos (aceite de maíz, aceite de semillas de girasol), los cuales son componentes lipídicos. La referencia D1 también divulga una forma de dosificación oral para el ABT-263 que comprende DMSO (10%) en PEG-400 en el párrafo [0145]. En particular, esta formulación ejemplificada carece de un vehículo lipídico.

Aunque la referencia D1 divulga antioxidantes entre varios otros excipientes en el párrafo [0099], la referencia D1 no divulga ni sugiere un antioxidante calcógeno más pesado farmacéuticamente aceptable, por ejemplo, metabisulfito de sodio. La referencia D1 además no divulga ni sugiere vehículos fosfolipídicos. La referencia D2 divulga liposomas los cuales se asegura que son adecuados para la administración de principios activos farmacéuticos hidrofóbicos. Los liposomas se preparan combinando un compuesto farmacéuticamente activo, fosfolípidos, triglicéridos y opcionalmente etanol. Estos componentes forman pequeñas partículas lipídicas al entrar en contacto con el agua las cuales encapsulan al agente activo.

La referencia D2 divulga y ejemplifica compuestos activos que son, cada uno o ambos, solubles en agua y/o de tipo fármaco por naturaleza, y por esto no poseen ninguna dificultad particular para formular un sistema de vehículo adecuado. Debido a que la referencia D2 divulga el éxito en formular un sistema de vehículo únicamente para compuestos tipo fármaco, una persona normalmente versada en la materia no podría simplemente haber predicho que el vehículo de la referencia D2 hubiese sido adecuado para usar con los compuestos tipo no fármaco de la reivindicación 1. En este sentido, la referencia D2 muestra la encapsulación de varios ingredientes activos, incluyendo vitamina B12, cianocobalamina, heparina (Fragmin), clorhidrato de buspirona y coenzima Q10. Aunque la vitamina B12 y la cianocobalamina tienen alto peso molecular, ambas tienen alta solubilidad en agua y alta permeabilidad. En consecuencia, cada uno de ellos es un compuesto de Clase I de acuerdo al Sistema de Clasificación Biofarmacéutica, y cada uno de ellos puede ser fácilmente formulado en una forma de dosificación oral adecuada. Como se demuestra en la referencia D2, el Fragmin también es altamente soluble en agua, ya que las composiciones de los Ejemplos allí mostrados, comprenden 120 mg de heparina por gramo de agua. La buspirona es también altamente soluble en agua y tiene un peso molecular pequeño de 385,5 g/mol. El último compuesto demostrado es la coenzima Q10, que es un fármaco de Clase II de acuerdo al Sistema de Clasificación Biofarmacéutica. En vista de lo anterior, una persona versada en la materia no hubiera podido reconocer que el vehículo de la referencia D2 era apropiado para compuestos farmacéuticos del grupo I y II del Sistema de Clasificación Biofarmacéutica.

Una persona normalmente versada en la materia no hubiese ni podría haber predicho que el vehículo de la referencia D2 hubiese sido adecuado para los compuestos reivindicados, los cuales son por naturaleza de tipo no fármaco y están clasificados en la Clase IV del Sistema de Clasificación Biofarmacéutica y por lo tanto son reconocidos por ser difíciles de formular en productos farmacológicos para administración oral. Dicho de otra manera, una persona normalmente versada en la materia no podría haber extrapolado el éxito demostrado para los vehículos de la referencia D2 para compuestos que se sabe que son fáciles de formular en formas de dosificación oral, a los compuestos de la reivindicación 1, los cuales se desvían del comportamiento tipo fármaco y son por lo tanto difíciles de formular en composiciones de administración oral.

En vista de la conocida dificultad para formular composiciones para administración oral adecuadas para compuestos de Clase IV (los cuales abarcan a los compuestos reivindicados) y la naturaleza única y sustancialmente de tipo no fármaco de los compuestos reivindicados, una persona normalmente versada en la materia no podría

haber predicho que los vehículos de la referencia D2, de los cuales sólo se demostró su uso con compuestos farmacológicos de Clase I y Clase II, hubiesen sido adecuados para formular una composición para administración oral para los compuestos de fórmula I. En vista de lo anterior, la divulgación del Solicitante como se revela en las presentes reivindicaciones posee actividad inventiva respecto al arte previo, y el rechazo debería ser retirado.

Todas las reivindicaciones que dependen de la reivindicación 1 son patentables por las mismas razones que la reivindicación 1. Las reivindicaciones independientes 10 y 30 y las reivindicaciones que dependen de ellas son también inventivas respecto a las referencias citadas por las razones mencionadas más arriba en conexión con la reivindicación 1. En vista de lo anterior, las reivindicaciones de la presente solicitud están en condiciones de ser concedidas.

En conexión con lo discutido anteriormente, me permito referirme al señalamiento que hace el Tribunal de Justicia de la Comunidad Andina con respecto a las definiciones de "obvio" y "evidente" en la evaluación del Nivel Inventivo de una solicitud de patente¹:

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

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

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

Dentro de este marco de ideas y a la luz de los argumentos expuestos en el presente memorial, me permito resaltar que al no "saltar a la vista de manera clara y directa" a partir de los documentos D1 y D2 la composición farmacéutica que posean

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

todas y cada una de las características esenciales de aquella que es objeto de la presente invención y al no tenerse "certeza clara y manifiesta" a partir de las enseñanzas de dichos documentos el diseño de una composición farmacéutica para administración oral adecuada para compuestos de Clase IV (los cuales abarcan a los compuestos reivindicados) y la naturaleza única, sustancialmente de tipo no fármaco de los compuestos reivindicados, es claro el Nivel Inventivo que conlleva la solicitud en estudio con respecto al arte previo citado.

6. **CONCLUSIÓN.**

Para finalizar me permito resaltar que el nuevo capítulo reivindicatorio presentado junto con este memorial es totalmente claro y conciso, al reclamar las realizaciones preferidas de la invención, cumpliendo así con lo exigido por la Decisión Andina 486 de 2000.

Por otro lado, en virtud de los argumentos discutidos con respecto a los documentos citados, me permito indicar que se estableció de manera incontrovertible que los compuestos que son objeto de la solicitud en estudio no están revelados en el arte previo y que la persona versada en la materia no podría obtener dichos compuestos de manera evidente a partir de las enseñanzas de los documentos citados.

7. Anexo al presente memorial me permito presentar:

- (a) Copia del artículo PAOLINI, Gaia V.; SHAPLAND, Richard H. B.; VAN HOORN, Willem P.; MASON, Jonathan S. & HOPKINS, Andrew. "Global mapping of pharmacological space". Nature Biotechnology, Julio 2006, vol 24, no 7, Págs. 805 – 815;
- (b) Copia del artículo LESSENE, Guillaume; CZABOTAR, Peter E.; COLMAN, Peter M. "BCL-2 Family antagonists for cancer therapy". Nature Reviews. Drug Discovery, diciembre 2008, vol 7, Págs. 989 – 1000; y
- (c) Copia de las páginas **110 a 121** que corresponde al **nuevo capítulo reivindicatorio**, discutido en el presente memorial, constituido por **37** reivindicaciones.

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

Señor Superintendente,



ALICIA LLORED A RICAURTE

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Global mapping of pharmacological space

Gaia V Paolini^{1,3,7}, Richard H B Shapland^{1,4,5}, Willem P van Hoorn^{2,3}, Jonathan S Mason^{3,6} & Andrew L Hopkins^{1,3,7}

We present the global mapping of pharmacological space by the integration of several vast sources of medicinal chemistry structure-activity relationships (SAR) data. Our comprehensive mapping of pharmacological space enables us to identify confidently the human targets for which chemical tools and drugs have been discovered to date. The integration of SAR data from diverse sources by unique canonical chemical structure, protein sequence and disease indication enables the construction of a ligand-target matrix to explore the global relationships between chemical structure and biological targets. Using the data matrix, we are able to catalog the links between proteins in chemical space as a polypharmacology interaction network. We demonstrate that probabilistic models can be used to predict pharmacology from a large knowledge base. The relationships between proteins, chemical structures and drug-like properties provide a framework for developing a probabilistic approach to drug discovery that can be exploited to increase research productivity.

The foundation for developing drug discovery into a knowledge-based predictive science lies, in part, in the assembly and integration of all medicinal chemistry structure-activity information¹. Although access to protein sequence data is widely available through global genome repositories, no such integrated databanks exist for medicinal chemistry structure-activity data. Public initiatives, such as the Harvard University (Cambridge, MA, USA) ChemBank Initiative², the US National Cancer Institute (Bethesda, MD, USA) Screening Database³ and the US National Institute of Mental Health's (Bethesda, MD, USA) Psychoactive Drug Screening Program K_i Database⁴, are important developments toward disseminating SAR data. However, most pharmacological data exists in proprietary screening databases, published documents, such as journal articles and patents, and a growing variety of commercial databases. The lack of accepted data standards and data integration thus prevents knowledge discovery and data-mining efforts from learning from the output of the significant annual private and public investment in pharmaceutical research.

To navigate chemogenomic knowledge space, we have created a comprehensive assembly of annotated pharmacological data³⁻⁸. We have also designed a unified data model to enable the global mapping and measurement of pharmacological space (that is, biologically active chemical space) by the integration of diverse data sources into a single data warehouse. Although a possible alternative to this would be a federated approach, we found that a single database model better fitted with our data-integration vision as well as with our practical, architectural and technical constraints. We applied the principle of knowledge discovery in databases to the design^{9,10}, including data conversion, cleaning and transformation. We found that having all the data in one place offers greater control for entity indexing and data retrieval and management, enabling us to perform global mapping. Ultimately, we believe that the implementation, although important, is a separate issue and it is the integration concept and the data model, however physically realized, that matter. The data are integrated by chemical structure, using unique canonical representations, including the often-neglected issue of tautomers. Assay data are assigned to targets by protein sequence, and indications indexed by a disease code. Thus, both chemoinformatics and bioinformatics techniques can be applied directly to the data-mining of the integrated data set.

At present, the data warehouse contains 4.8-million nonredundant chemical structures, over 275,000 of which are classified as biologically active. Over 600,000 SARs of molecular binding (e.g., IC₅₀; inhibitor concentration required for 50% inhibition of the normal reaction) data from Pfizer's internal screening files are integrated with commercial screening data, competitive intelligence on approved and investigational drugs and key components of the past 25 years of published medicinal chemistry data.

Pharmacological target space

Large-scale data integration of proprietary and published screening data enables the identification of the number of unique molecular targets, as represented by protein sequences, for which chemical tools, leads or drugs have been discovered. Because of the lack of integrated knowledge bases in pharmaceutical research, the list of molecular targets for which small-molecule chemical matter has been discovered has been difficult to ascertain¹¹⁻¹⁴. We have assigned 2,876 targets to protein sequences from 55 organisms, with biologically active chemical tools for 1,306 proteins. However, because of orthologs among species, many of the mammalian genes are redundant.

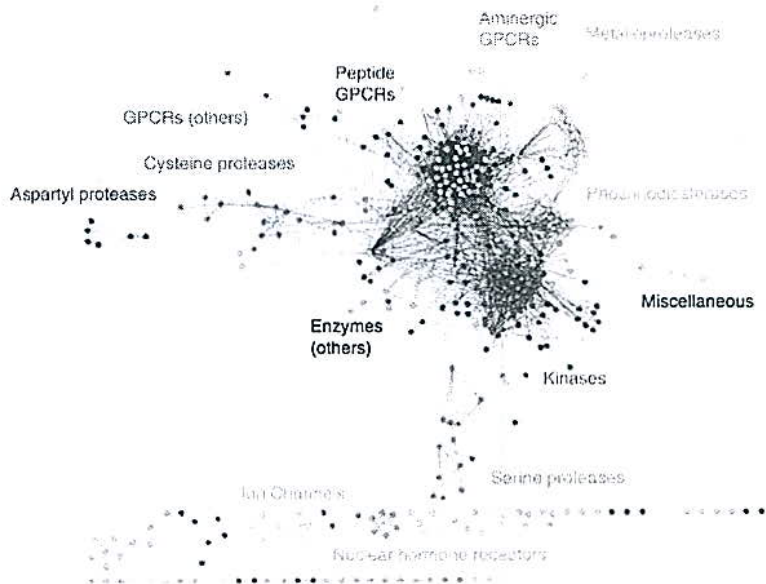
In total, we can unambiguously identify 836 genes in the human genome for which small-molecule chemical tools have been discovered (the threshold of biological activity is defined throughout as a binding affinity <10 μ M). When Lipinski's rule-of-five criteria for oral drug

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Figure 1 Human polypharmacology interaction network representing relationships between proteins in chemical space. Two proteins are deemed interacting in chemical space (joined by an edge) if both bind one or more compound within a defined difference in binding energy threshold ($n = 3$ in this plot, see Methods section). The number of proteins in this network is 486 (nodes), with 3,636 polypharmacology relationships (edges), where the $P_{ij} > 0.1$ (P_{ij} is defined in the Methods section), the number of shared compounds between two proteins is >1 and the number of cotested compounds for two targets is $N_{ij}^{\text{tested}} > 10$. Nodes are colored by gene family.



absorption¹⁵ are applied, 727 human targets have at least one compound with a binding affinity $<10 \mu\text{M}$ and 529 human targets have at least one compound with a binding affinity $<100 \text{ nM}$ that satisfy the rule-of-five (Table 1). Of the pharmacological targets selected, 158 human proteins have been identified as the primary modes-of-action for approved small-molecule drug targets with oral small-molecule drugs primarily targeting only 141 human proteins.

Polypharmacology

A key question in global pharmacological space is how extensive is promiscuity, which is defined as the specific binding of a chemical to more than one target. Considering each pair of targets in turn, if two proteins both bind to the same ligand, they can be considered as interacting in chemical space, even if they have no other interaction in physical space or similarity in sequence space. The concept of ‘target-hopping,’ where chemical matter for one

target can be considered as the basis for leads or tools for another target has historically been an extremely fruitful method of drug discovery^{16–18}. The entire database was analyzed to ensure that nonspecific aggregation inhibitors¹⁹ did not bias the results. Of all the 276,122 active

Table 1 Pharmacological target space^a

Gene taxonomy	All targets with $<10 \mu\text{M}^a$ binding affinity	Human targets with $<10 \mu\text{M}$ binding affinity	Human targets with $<1 \mu\text{M}$ binding affinity	Human targets with $<10 \mu\text{M}$ binding affinity and rule-of-five ^b $N > 1$	Human targets with $<100 \text{ nM}$ binding affinity	Human targets with $<100 \text{ nM}$ binding affinity and rule-of-five ^b $n > 1$
Protein kinases	131	105	99	98	89	83
Peptide GPCRs	110	63	59	59	55	42
Transferases	75	49	42	36	33	24
Aminergic GPCRs	72	35	35	35	35	35
GPCRs (class A and others)	68	44	44	40	38	32
Oxidoreductases	68	40	36	38	29	25
Metalloproteases	63	44	41	41	36	35
Hydrolases	56	36	29	30	25	21
Ion channels (ligand-gated)	55	29	28	24	25	22
Nuclear hormone receptors	47	24	24	22	23	19
Serine proteases	37	30	30	28	29	21
Ion channels (others)	24	18	16	16	13	11
Phosphodiesterases	23	19	19	19	18	18
Cysteine proteases	20	16	16	14	14	13
GPCRs class C	20	10	10	10	6	6
Kinases (others)	16	12	9	11	6	5
GPCRs (class B)	14	7	7	4	7	3
Aspartyl proteases	10	7	7	4	6	4
Miscellaneous	241	139	119	108	83	63
Enzymes (others)	156	109	97	90	69	47
Total	1,306	836	767	727	639	529

^aOf the 1,306 targets with biological-active chemical tools or drugs in the database, only 131 targets have more than 1,000 active compounds, 299 targets have between 100 and 1,000 bio-active molecules and 761 targets have between 1 and 100 reported active compounds. In the analysis 115 targets were found with only one reported chemical tool, to date. ^bCompounds passing Lipinski's 'rule-of-five' criteria¹⁵ of fewer than 5 H-bond donors, fewer than 10 H-bond acceptors, MW below 0.5 kDa and clog P below 5. Compounds that fail Lipinski's criteria are more likely to show poor absorption or permeation because such compounds are unlikely to show good oral bioavailability.

compounds found in our database, 65% have recorded activity for one target, whereas 35% are observed to hit more than one target.

We have mapped the observed polypharmacology interaction network for human proteins (Fig. 1) to navigate polypharmacology relationships between targets. Each node of the network is a human target for which we found active lead matter. Two nodes are connected if they share active matter. The strength of this connection (P_{ij}) is defined in the Methods section. Calculation of the polypharmacology network enables the visualization of the interactions between proteins in chemical space. The entire protein interaction network for human proteins, calculated from our database, consists of 700 proteins (nodes) connected by 12,119 interactions (edges) for all compounds below the affinity threshold of 10 μ M and with a difference in affinity of up to three orders of magnitude between two targets. Interestingly, the structure of the network is robust to changes in the window of fold-differences in affinity; 696 proteins (nodes) are connected by 11,591 interactions (edges) for all compounds with an affinity threshold <10 μ M that have a difference in affinity of up to two orders of magnitude between two targets, and 675 proteins (nodes) are connected by 10,016 interactions (edges) for all compounds with an affinity threshold <10 μ M that have a difference in affinity of up to one order of magnitude between two targets. We should stress, however, that the SAR matrix is far from complete, and new data becoming available could alter the appearance of the network, as noted by Vieth *et al.*²⁰.

Promiscuity can be considered from the perspective of both the compound and the pharmacological target, to measure compound selectivity and target overlap^{20–22}. We evaluated the degree of promiscuity of each target in three different ways (see Methods section for definitions). Table 2 shows the top ten promiscuous targets obtained using the different methods. Method one (P_1) consists of calculating a target's promiscuity as the proportion of ligands shared with other targets, multiplied by the average number of targets that each of the target's ligands is active against. This definition promotes targets whose ligands are predominantly promiscuous, with a high number of other targets. The second method (P_2) uses the polypharmacology network. This promiscuity index is calculated by counting the number of connections of each target (edges connected to each node in the network). This definition promotes targets that are connected to a large number of other targets, regardless of the strength of the interaction. The third definition (P_3) again uses the polypharmacology network, but this time the strength of the connections (P_{ij}) is used in the summation. It is apparent that the different definitions of promiscuity highlight different effects, although the same target classes (aminergic G protein-coupled receptors (GPCRs), cytochrome P450s and protein kinases) appear at the top positions (Table 2). By comparing the rankings of targets resulting from using P_1 , P_2 and P_3 , we find that P_1 is correlated with neither P_2 nor P_3 ($R < 0.5$) whereas

Table 2 Most promiscuous human proteins calculated using P_1 , P_2 and P_3 promiscuity indexes^a

Order	Target	Gene family	P_1 value	N active compounds
P_1 promiscuity index				
1	Histamine H2 receptor	Aminergic GPCRs	10.6	372
2	Cytochrome P450 3A5 (nifedipine oxidase)	Enzymes (others)	8.6	148
3	Cytochrome P450 2D6 (debrisoquine 4-hydroxylase)	Enzymes (others)	8.4	416
4	Cytochrome P450 2C19 (S-mephenytoin 4-hydroxylase)	Enzymes (others)	6.5	194
5	Imidazoline (I-1) receptor candidate	Miscellaneous	5.0	140
6	Muscarinic acetylcholine receptor M5	Aminergic GPCRs	4.9	152
7	Alpha-2B adrenergic receptor	Aminergic GPCRs	4.8	685
8	Muscarinic acetylcholine receptor M4	Aminergic GPCRs	4.8	420
9	Cytochrome P450 2C9 (CYP2C9)	Enzymes (others)	4.7	200
10	Protein kinase C delta type (NPKC-delta)	Protein kinases	4.4	188
P_2 promiscuity index				
			P_2 value	
1	D(2) dopamine receptor	Aminergic GPCRs	112	8,840
2	5-hydroxytryptamine 1A receptor (5HT1A)	Aminergic GPCRs	107	8,763
3	5-hydroxytryptamine 2C receptor (5HT2C)	Aminergic GPCRs	105	8,051
4	Cytochrome P450 3A4	Enzymes (others)	104	3,549
5	Histamine H1 receptor	Aminergic GPCRs	93	2,896
6	5-hydroxytryptamine 2A receptor (5HT2A)	Aminergic GPCRs	92	7,849
7	Alpha-1A adrenergic receptor	Aminergic GPCRs	89	4,024
8	5-hydroxytryptamine 7 receptor (5HT7)	Aminergic GPCRs	87	2,774
9	Cytochrome P450 1A2 (P-3-450)	Enzymes (others)	87	10,719
10	Muscarinic acetylcholine receptor M1	Aminergic GPCRs	84	1,567
P_3 promiscuity index				
			P_3 value	
1	Cytochrome P450 1A2 (P-3-450)	Enzymes (others)	27.3	10,719
2	5-hydroxytryptamine 2C receptor (5HT2C)	Aminergic GPCRs	19.3	8,051
3	Cytochrome P450 3A4	Enzymes (others)	19.2	3,549
4	D(2) dopamine receptor	Aminergic GPCRs	18.5	8,840
5	SRC kinase	Protein kinases	17.7	1,749
6	5-hydroxytryptamine 1A receptor (5HT1A)	Aminergic GPCRs	17.1	8,763
7	5-hydroxytryptamine 2A receptor (5HT2A)	Aminergic GPCRs	16.1	7,849
8	D(4) dopamine receptor	Aminergic GPCRs	15.8	2,709
9	Alpha-1A adrenergic receptor	Aminergic GPCRs	14.5	4,024
10	5-hydroxytryptamine 7 receptor (5HT7)	Aminergic GPCRs	14.3	2,774

^aIn evaluating P_1 , only proteins with over 100 biologically active compounds were included. In evaluating P_2 and P_3 , only network connections with a number of commonly tested compounds greater than 10 were included. Promiscuity indices P_1 , P_2 and P_3 are defined in the Methods section.

P_2 and P_3 are strongly correlated ($R = 0.9$). This is consistent with the fact that P_2 and P_3 are calculated using the same network, but also supports the view that connectivity, regardless of the relative strengths of the connections, is the important ingredient in the structure of the polypharmacology interaction network.

The majority of compounds are active against targets within the same gene family. However, as we observed from the structure of the polypharmacology interaction network, there is significant interaction between gene families. A quarter of all the promiscuous compounds have been observed to be active across different gene families. To visualize the polypharmacology interactions at gene-family level, we have summarized the target-target interaction network by summing all the P_{ij} values by gene family (G_{kb} , see Methods sections). The resulting matrix is shown in Figure 2 (see data supplied in Supplementary Table 1 online). Using this matrix, we can illustrate the cumulative strength of intra- as well as inter-gene family connections, the latter represented by the off-diagonal cells. Aminergic GPCRs and protein kinases exhibit the greatest intra- as well as inter-gene family promiscuity.

Bayesian predictions of pharmacology

We decided to investigate the construction of a virtual array of predictive pharmacology models derived from the analysis of the large-scale integrated SAR data. Using a Laplacian-modified Bayesian classifier approach^{23,24}, 698 target-specific predictive models were built. All the compounds classified as biologically active in the database were filtered by chemical quality criteria. Of the remaining compounds, 10% were removed for the test set (23,792 compounds with 55,781 measurements) and 90% of the data (214,128 compounds with 561,913 measurements) were used to build the predictive models. The Bayesian model for each target was built using the training set where all compounds are classified as either active (endpoint < 10 μ M for that target) or inactive (the rest). A Bayesian model prediction is a number describing confidence of activity: the larger the score, the more confidence the compound is active, but no quantitative prediction of affinity is made. Similarly, a large negative score indicates high confidence of inactivity, and finally, a score close to zero is a neutral prediction. Bayesian prediction scores for all test set compounds were calculated across the bank of 698 models.

The success rates of the combined predictive models above the random baseline prediction are shown in Figure 3 (see data in Supplementary Table 2 online). All Bayesian scores greater than or equal to the cutoff are interpreted as predictions of activity. For example, at the confidence score cutoff of 50, 72% of compounds in the test set have at least one prediction, and 64% have at least one correctly predicted target in common with an experimental target, whereas only 4% were incorrectly predicted. At the Bayesian score of 50, a total of 58,428 biological activities are predicted, 56.7% of which are correct, representing a 153-fold enrichment over random. The predicted false-negative rate is 13%, whereas 26,828 false positives are apparently predicted. As the measured ligand-target matrix is only 0.4% full, many of the false-positive predictions may indeed still be true.

In addition to predicting primary pharmacology, we wanted to ascertain whether the models could be used to predict polypharmacology. To explore this problem, we have done a preliminary investigation with Cerep's (Paris) 'BioPrint' data set, which is a nearly complete matrix of measured activities of 997 compounds against 316 targets. Results of these studies can be found in the **Supplementary Figures 1 and 2 online** and **Supplementary Tables 3 and 4 online**. These initial studies indicate that probabilistic models built from integrated medicinal chemistry SAR data are a promising approach for predicting primary pharmacology across a large number of protein targets. In terms of polypharmacology, intra-gene family promiscuity is predicted with the highest confidence. Inter-gene family interactions are a much harder problem because of the sparse nature of the ligand-target matrix.

Relationship between molecular properties and target class

We calculated a set of physicochemical descriptors for all compounds in the database to investigate the relationship between target class and the physicochemical properties of ligands^{13,25}. The protein sequences assigned to each of the pharmacological targets were classified into gene families. Distinct differences in the distribution of molecular properties between sets of compounds active against different gene families are observed (Table 3, Fig. 4 and Supplementary Fig. 3 online). For example, the mean molecular weight (MW) of ligands binding to aminergic GPCRs is 378 Da (s.d. = 93 Da), whereas the mean MW of peptide GPCR ligands is greater at 514 Da, but with a wider spread (s.d. = 202 Da). Ligands for the nuclear hormone receptors are the most lipophilic, as measured by calculated octanol/water partition coefficient (clogP), mirroring the properties of steroids. Overall, the properties of the synthetic ligands reflect the differences in the properties of the endo-genous ligands for each target class.

The distribution patterns illustrate that, although there are distinctions in the physical properties of the ligands, using a single property to discern separate gene families is too crude. We wanted to investigate whether ligands for specific gene families may be selected within a range of property parameters. Using a 184,687-compound subset of the data as a training set, linear discriminant analysis was used to classify 41,823 compounds by target class using only the calculated physicochemical molecular properties. The data set used for the linear discriminant analysis consisted of the subset of compounds that bind to members of exactly one target class. Overall, this simplistic method successfully classified 34% of ligands to their respective target classes, with an overall enrichment ratio over random of 6.9 (Supplementary Table 5 online). The results are interesting as they suggest that simple calculated molecular properties can be used as a crude classifier of a compound's biological activity, by gene family.

Industrial trends of compounds, targets and attrition

We have witnessed a remarkable growth in the number of reported targets and compounds disclosed in the medicinal chemistry literature, mirroring the rise in investment in pharmaceutical research. In recent years, the number of targets

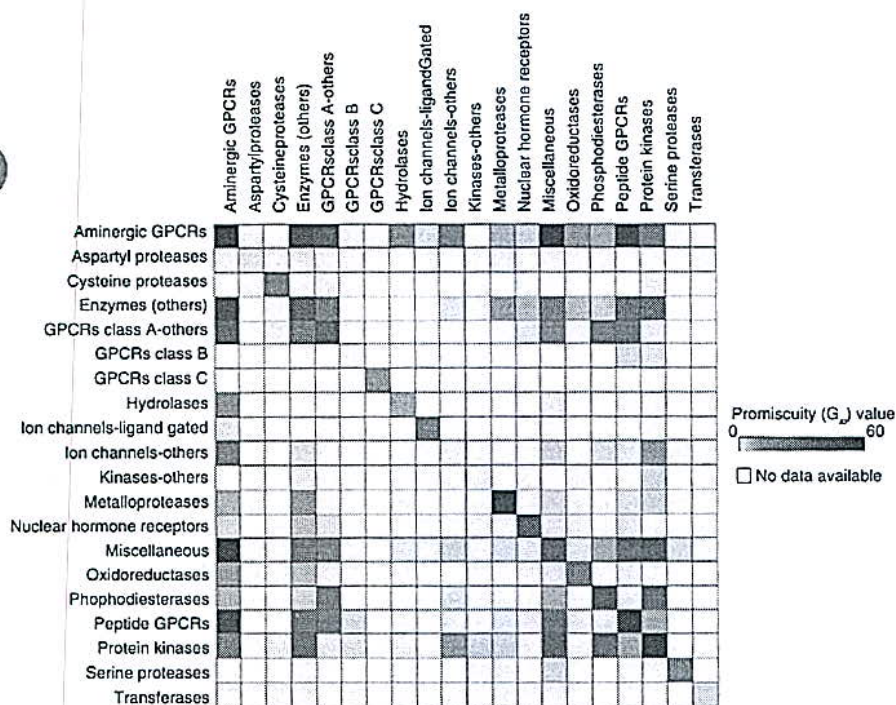


Figure 2 Degree of intra- and inter-gene family promiscuity illustrated as a polypharmacology interaction matrix. The degree of promiscuity, as measured by G_M , is color-coded. White cells represent lack of information. The number of target pairs used in the summation and the level of compound statistics are shown in Supplementary Table 1 online.

screened, including selectivity counter-screens, published in the medicinal chemistry literature, has been growing drastically. Screening data on nearly 900 proteins are currently published each year, of which >500 molecular targets are reported with potent chemical matter (that is, $IC_{50} < 100$ nM). Currently, potent novel chemical tools and leads are first disclosed for ~80–100 new molecular targets each year (Fig. 5a). No doubt, this is a conservative estimate as many new compounds and targets are only disclosed in patents, which are not included in this initial literature analysis. The increase in the rate of discovery of chemical tools for new targets doubled from an average of 30 new targets with leads being disclosed in the 1980s to an average of 60 new targets per year in the 1990s. In comparison, an average of four new targets, for first-in-class drugs, have reached the market each year during the 1990s¹³.

That said, we have yet to see the increase in new targets with leads translating into a proportionate increase in the number of approved first-in-class drugs. An analysis of the targets of published compounds reveals some significant trends in the changing character of the industry's portfolio of targets and target classes (Fig. 5b), such as a relative decline in proportion of aminergic GPCRs in the industry's target portfolio and an increase in protein kinases.

Over the past 25 years, there has been a steady, inexorable rise in the median MW of reported medicinal chemistry compounds (Fig. 5c). Comparing 5-year averages from 1986–1990 to those of 1999–2003, the median MW of all reported medicinal chemistry compounds in the literature rose 68 Da (~20%) from 354 Da to 422 Da, respectively. Interestingly, this growth is also reflected in the increase of the median MW of disclosed ligands for several gene families. For example, compounds binding to aminergic GPCRs have increased in MW by around 56 Da, from 337 Da to 393 Da between the two 5-year periods. No significant increase in mean or median potency is observed in the data to explain the increase in MW. Even so, this rise in MW contrasts with the steady state of the mean MW of approved drugs²⁶ and the steady decline in MW through each subsequent stage of clinical development and increase in the proportion of compounds that are rule-of-five compliant^{27,28} (Fig. 5d).

Of course, these calculations combine all target classes together; in contrast, the industry's target portfolio is unlikely to be in a steady state, with some target classes emerging and others declining in popularity. The relative difference in molecular properties among the gene families is also reflected in compounds in clinical development; however, again we notice that, even within a gene family, the median MW of compounds surviving subsequent clinical phases is declining slightly (Fig. 5d).

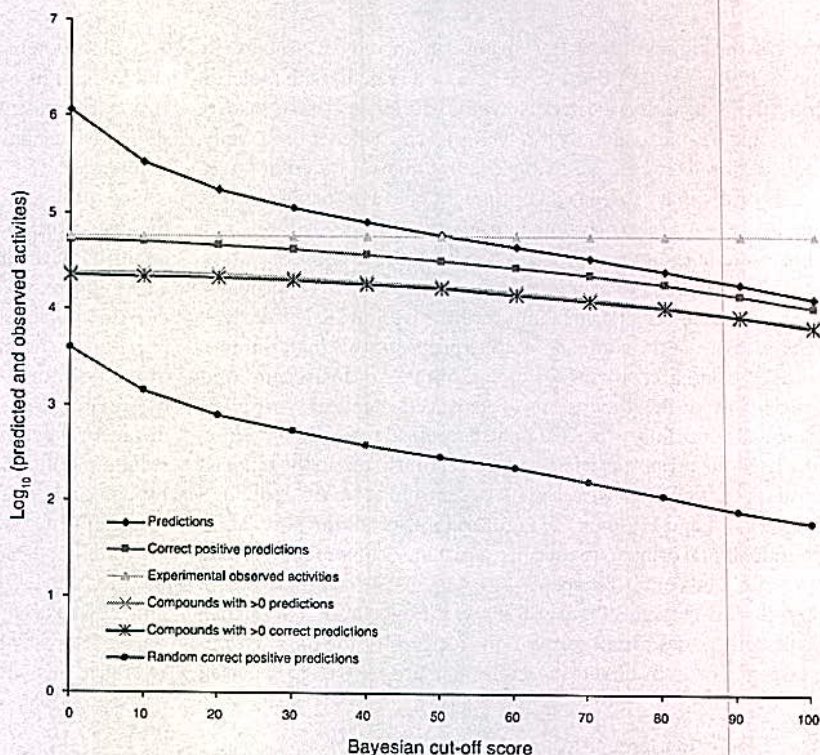


Figure 3 Bayesian predictions of pharmacology. Relationship between Bayesian confidence levels and number of predictions from 23,792 compounds with 55,781 measured biological activities across 698 activity models.

Degrees of druggability

A key objective of our global analysis of pharmacological space is to build the foundation of probabilistic approaches to drug discovery. Trends from marketed and investigational drugs indicate that oral drug space

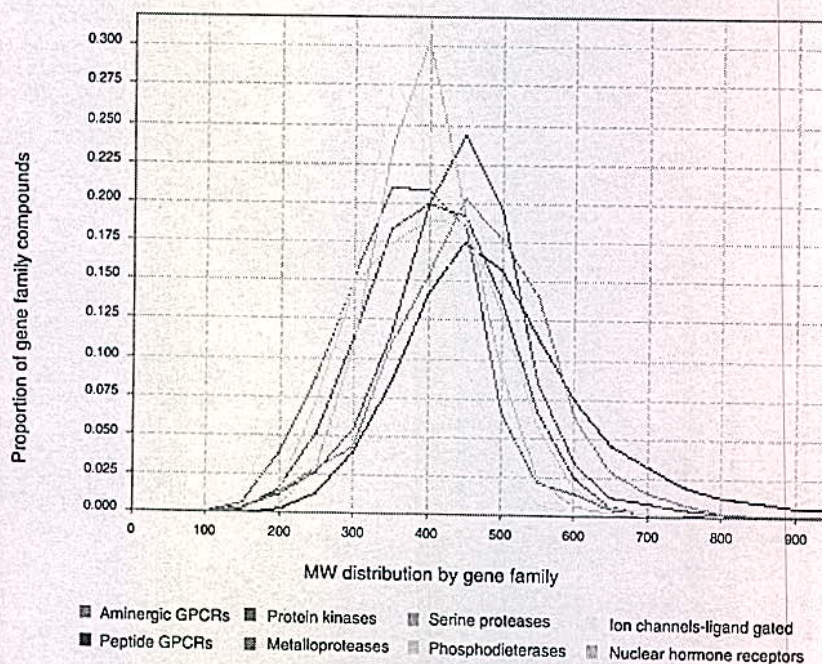


Figure 4 Molecular weight (MW) distribution of compounds by gene family. See Supplementary Figure 3 for property of MW, cLogP, number of rotatable bonds and number of hydrogen-bond acceptors, by gene family.

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is limited by the biophysical barriers to absorption and permeability in the human body^{15,26,29–40}. Because we have observed that the molecular properties of ligands are correlated with their target class, it follows that we should be able to identify those targets with a higher probability to produce drug-like chemical matter. Rather than considering target druggability as a binary state, it can be thought of as a probabilistic continuum, where two targets may both be classified as druggable¹³ but may exhibit considerable differences in their probabilities of success.

Lipinski introduced the concept of upper physicochemical property limits, above which drug permeability and absorption are less likely¹⁵. Like Lipinski, we use the simple molecular properties of clogP, number of hydrogen-bond acceptors (H-acc) and number of hydrogen-bond donors (H-don) as the dimensions of a reduced chemical space. Oral drugs are still the primary focus of pharmaceutical research; therefore, we calculated the properties of 617 approved oral drugs in the reduced chemical space (for which we calculated a centroid at MW = 316, clogP = 2.3, H-acc = 4 and H-don = 2). Figure 6a illustrates the population distribution of oral drugs in two-dimensional molecular property space as an interpolated contour map.

In terms of drug targets Figure 6b shows the distribution of median molecular properties for all compounds for each of the human oral-drug targets. For each target, the molecular properties are averaged

over all its potent active compounds (<100 nM), including oral drugs and leads. Figure 6c shows the same quantities, this time for all human targets with potent active compounds. Comparison of these two figures shows that a significant number of targets are outside the rule-of-five boundaries.

Given the set of active compounds observed for a target, could the ligand properties in reduced chemical space provide a guide to quantifying the likelihood of the target to produce an oral drug? As a first approximation, the degree of druggability of the target can be described as the distance D_T between the target T and the oral drugs, in reduced chemical space. This distance is expressed as a function of the deviation of the centroid of each target from the ideal value of the oral-drugs distribution (see Methods section). The resulting distance ranges from 0 to 1, with ideal value being 0. If we compare the results for all human targets (excluding known drug targets) versus human oral drug targets, we observe an enrichment in the degree of druggability of drug targets versus all the remaining human targets. We find that 87% of human oral-drug targets have $D_T \leq 0.6$, and 65% have $D_T \leq 0.4$. Of the remaining human targets, 68% have $D_T \leq 0.6$, and 39% have $D_T \leq 0.4$. This means that ~200 of the remaining targets have a relatively high degree of druggability ($D_T \leq 0.4$), but have yet to realize this potential.

Table 3 Molecular properties of gene family ligands

Gene taxonomy	MW (Da) (mean)	MW (Da) (s.d.)	MW (Da) (median)	90% limit of MW (Da)	clogP (mean)	clogP (s.d.)	clogP (median)	90% limit of clogP
Aminergic GPCRs	378	93	376	460	3.8	1.6	3.9	5.6
Ion channels (ligand-gated)	359	91	362	430	3.0	1.8	3.2	4.7
Metalloproteases	428	103	429	530	3.0	1.9	3.1	4.8
Nuclear hormone receptors	398	96	396	495	5.1	1.7	5.0	7.3
Peptide GPCRs	514	202	477	752	4.3	2.3	4.6	6.5
Phosphodiesterases	400	65	397	465	3.7	1.4	3.7	5.2
Protein kinases	407	109	402	505	3.8	1.8	3.9	5.7
Serine proteases	467	145	463	572	2.7	2.1	2.7	4.8

Gene taxonomy	Number of hydrogen bond acceptors (Mean)	Number of hydrogen bond acceptors (SD)	No. hydrogen bond acceptors (Median)	90% limit of number of hydrogen bond acceptors	Number of hydrogen bond donors (Mean)	Number of hydrogen bond donors (SD)	Number of hydrogen bond donors (Median)	90% limit of number of hydrogen bond donors
Aminergic GPCRs	4	2	4	6	1	1	1	2
Ion channels (ligand-gated)	4	2	4	6	2	1	2	3
Metalloproteases	6	2	6	8	3	1	2	4
Nuclear hormone receptors	4	2	4	6	1	1	1	2
Peptide GPCRs	5	4	4	10	2	3	1	8
Phosphodiesterases	6	2	6	8	1	1	1	2
Protein kinases	5	2	5	7	2	1	2	4
Serine proteases	5	3	5	8	3	2	2	4

Gene Taxonomy	Number of rotatable bonds (mean)	Number of rotatable bonds (s.d.)	Number of rotatable bonds (median)	90% limit of number of rotatable bonds	Ligand efficien- cy ^a (kcal/mol/ non-H atom) (mean)	Ligand efficien- cy ^a (kcal/mol/ non-H atom) (s.d.)	Ligand efficien- cy ^a (kcal/mol/ non-H atom) (median)
Aminergic GPCRs	6	3	6	8	0.4	8.0E-02	0.4
Ion channels (ligand gated)	5	3	4	7	0.4	0.1	0.4
Metalloproteases	8	4	8	13	0.4	0.2	0.3
Nuclear hormone receptors	6	3	6	10	0.3	6.E-02	0.3
Peptide GPCRs	9	7	8	17	0.2	7.E-02	0.2
Phosphodiesterases	6	3	6	9	0.3	3.E-02	0.3
Protein kinases	6	3	5	9	0.3	7.E-02	0.3
Serine proteases	8	5	7	12	0.3	9.E-02	0.3

^aLigand efficiency⁵¹ of each compound in the gene family. The ligand efficiency is defined as the binding energy per atom⁵² ($\Delta G/n$, where n is the number of nonhydrogen (non-H) atoms).

DISCUSSION

The large-scale integration of medicinal chemistry and pharmacological data enables for the first time the global surveying and navigation of the biologically active chemical space (pharmacological space). Our initial investigations illustrate how the pharmacological target space of potential drug targets is a function of the physicochemical property filters applied to the ligands^{13,25}. The number of proteins for which chemical tools has been identified is significantly higher than previous estimates^{11–13}. The compilation and dissemination of chemical tools identified in a global survey, such as this, could be the basis of a rich chemical toolbox for chemogenomics^{7,25,41,42}, providing that the proper legal safeguards and respect for intellectual property are observed.

The comprehensive cataloging of biologically active chemicals also fosters the development of systematic ontologies for pharmacology and medicinal chemistry^{43,44}. The concept of relating proteins in chemical

space by polypharmacology interactions provides the foundation for a ligand-based protein classification and valuable resource for understanding the molecular basis for compound promiscuity^{5,45,46}. Our initial focus has been on *in vitro* binding and selectivity data. Although molecular data provide us with invaluable insights into molecular recognition, ultimately they need to be integrated with gene expression and phenotypic end-points from *in vivo* and clinical observations if we are to capture the relationships between molecular binding across the proteome with efficacy or toxicity.

In our opinion two interesting potential applications of this work are polypharmacology and probabilistic modeling. The mapping of polypharmacology networks enables us to start considering the rational design of selectively promiscuous agents, thereby expanding the opportunity space for new medicines. Approaching drug discovery as a probabilistic enterprise based on a priori knowledge with an understanding of

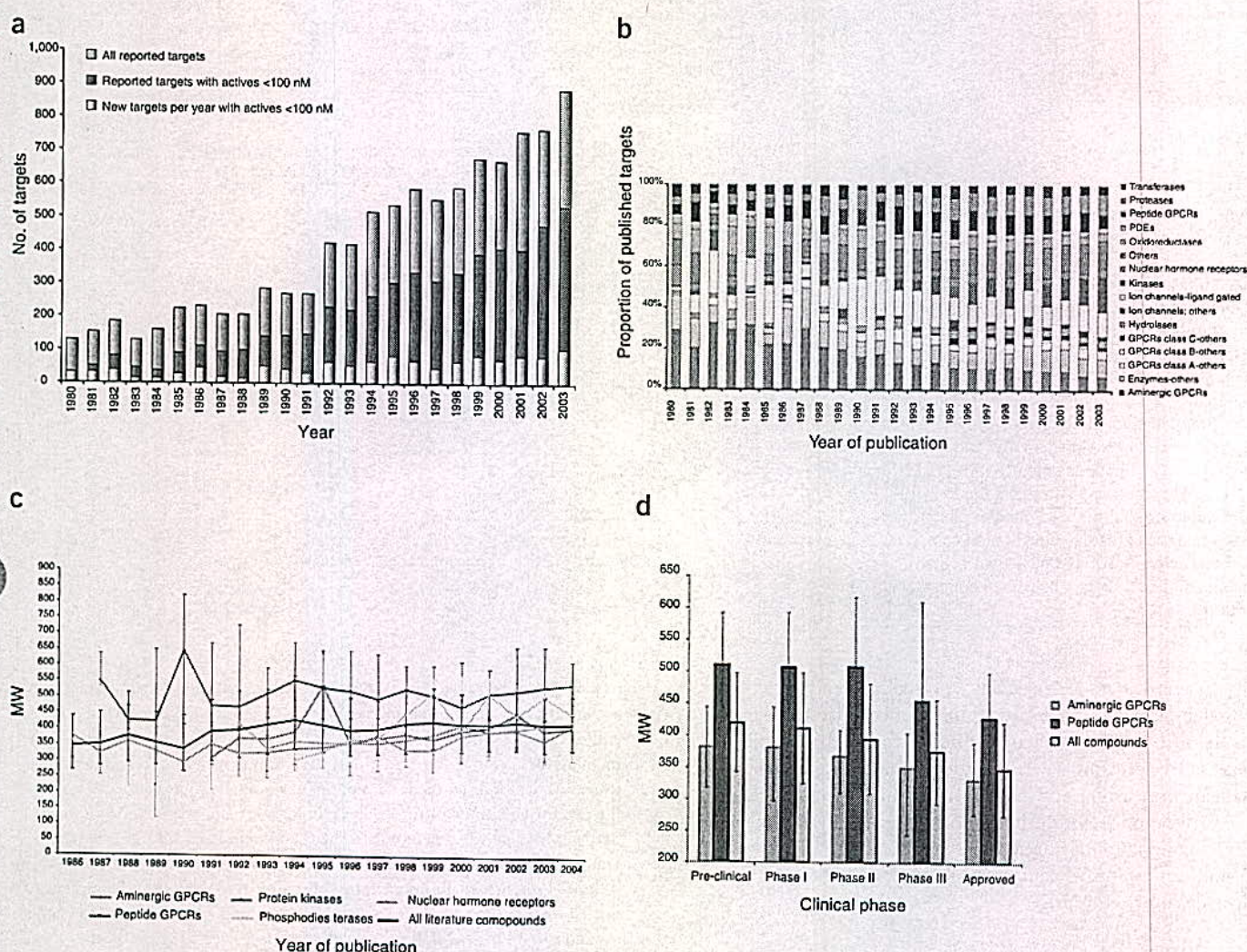


Figure 5 Trends in medicinal chemistry of compounds in the database. (a) Targets and new targets disclosed in the literature per year. (b) Changes in pharmaceutical industry's target portfolio, over time as derived from the published literature. (c) Increase in molecular weight over time of published compounds. (d) Changes in relative median MW among aminergic GPCRs, peptide GPCRs and all compounds through subsequent stages of clinical development. The number of launched drugs is the world wide approved count, irrespective of route of administration. The number of compounds in clinical stages are as follows. Total number of drugs in Phase I, 930; Phase II, 1,248; Phase III, 389; Approved, 1,631. Number of aminergic GPCR drugs in Phase I, 83; Phase II, 136; Phase III, 41; Approved, 185. Number of peptide GPCR drugs in Phase I, 53; Phase II, 100; Phase III, 17; Approved, 35. *t*-tests between aminergic GPCRs and peptide GPCRs indicate that the inter-gene family differences in MW are statistically significant with probability that the difference is due to chance $P < 0.0001$, as is the overall decline in MW of aminergic GPCRs and all compounds between preclinical and approved phases. The decline of peptide GPCRs between these two phases is significant with $P \sim 0.009$.

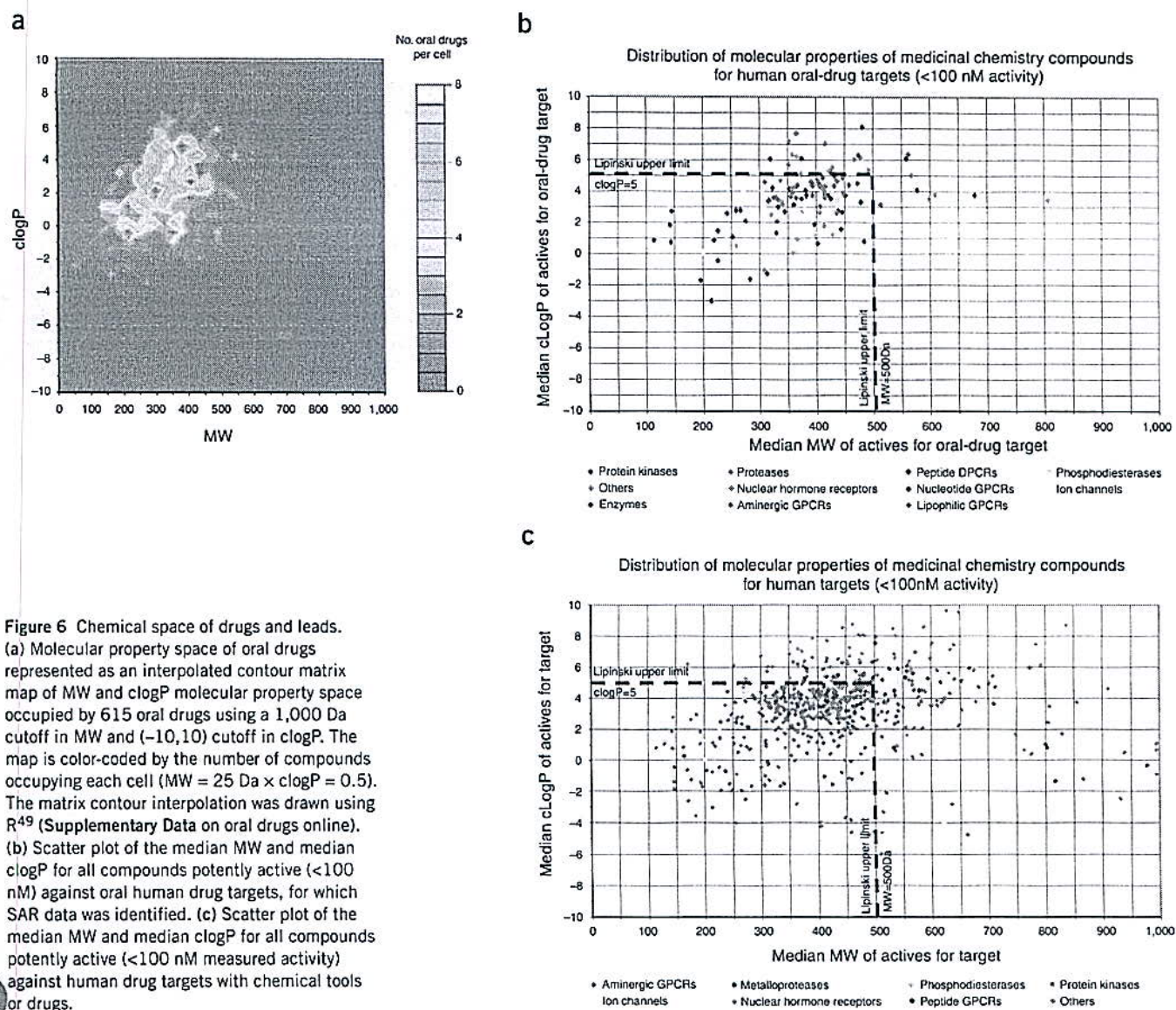


Figure 6 Chemical space of drugs and leads. (a) Molecular property space of oral drugs represented as an interpolated contour matrix map of MW and clogP molecular property space occupied by 615 oral drugs using a 1,000 Da cutoff in MW and (-10,10) cutoff in clogP. The map is color-coded by the number of compounds occupying each cell (MW = 25 Da × clogP = 0.5). The matrix contour interpolation was drawn using R49 (Supplementary Data on oral drugs online). (b) Scatter plot of the median MW and median clogP for all compounds potentially active (<100 nM) against oral human drug targets, for which SAR data was identified. (c) Scatter plot of the median MW and median clogP for all compounds potentially active (<100 nM measured activity) against human drug targets with chemical tools or drugs.

the varying degrees of druggability, promiscuity and attrition risks may be a significant advance in attempting to increase research productivity. As the vast majority of all drug discovery projects and clinical candidates fail the exacting criteria for safe human medicines, what we are left with are the learning and data that can contribute to the refinement of predictive models, for the benefit of all. Realization of the importance of the integration of our accumulated data can provide the basis for a significant improvement in our knowledge of success factors in the drug discovery enterprise.

METHODS

Database and data model. Our physical database consists of a single central Oracle 9.2 data warehouse. We store chemical structures as Simplified Molecular Input Line Entry Specification (SMILES) strings (<http://www.daylight.com>) and we use the Daylight DayCart Oracle Cartridge (<http://www.daylight.com/>) for structure indexing and manipulation. We chose SMILES as a database-friendly representation, as it is a compact, simple character syntax, encoding a self-contained language with its own controlled vocabulary and enabling unique canonical representations of structures, in which stereochemical descriptions can easily be defined or relaxed when querying structural data. Our data model is fully normalized to avoid bias

toward specific data queries. It is chemo-centric, in that we use chemical structures as the key to information storage and retrieval. This means that all the different entity types are ultimately connected to chemical structures. We are aware that methods for chemical representation are not fully mature and sometimes subjective. Consider for instance the perception of tautomeric equivalence (for example, the two unsubstituted nitrogens in an imidazole ring, one of which nominally needs to have a hydrogen atom attached) and tautomeric relations (where one tautomer may be considered more stable and thus the preferred drawing form or indeed where the tautomeric forms are considered to require chemical transformation). As a consequence, related software and rules are likely to change, expand and improve with time. For this reason we have designed the data model to handle multiple concurrent representations (that is, multiple SMILES strings) for any given compound. This way a fully flexible view of chemical structures and their connections can be achieved. We produced a single unified data warehouse integrating, by chemical structure, protein sequence and indication, the Pfizer's structure-activity data (e.g., IC_{50} , EC_{50} (concentration of a compound where 50% of its effect is observed), K_i , K_d excluding high-throughput screening percentage inhibition data), which contains data from legacy Pfizer (New York), Warner-Lambert (formerly of Morris Plains, NJ, USA, now part of Pfizer) and Pharmacia (formerly of Kalamazoo, MI, USA now part of Pfizer); the Inpharmatica (London) StARLite database, which contains data extracted from *Journal of Medicinal*

Chemistry (issues January 1980–Sept 2004) and *Bioorganic Medicinal Chemistry Letter* (issues January 1991–September 2004); the Cerep BioPrint database⁵ and summary data from the Thomson (New York) Current Drugs Investigational Drugs Database (IDDB) (<http://scientific.thomson.com/products/iddb/>). The current database contains 4.8-million unique chemical structures with protein identifiers and sequences assigned to 2,876 targets with assay measurements; 526,548 assay measurements are related to 276,122 active chemical structures.

Extraction, transformation and loading (ETL). Before being fully integrated into our database, the original data sources were first loaded into Oracle staging tables. This was achieved using a combination of tools (Servefile's Java-based data loader, Oracle, Pipeline Pilot). The staging tables were processed to perform data selection, cleaning, mapping and standardization. This ETL procedure is the most critical and time-consuming part of knowledge discovery in databases involving a blend of disciplines, namely scientific-domain expertise, logic and informatics. Data fields from the different data sources were identified and selected. Metadata tables were created to map together different conceptualizations of the same entities (that is, different ontologies). Data quality issues, ranging from spelling mistakes to entity misassignment, were addressed and contained or flagged. Chemical structures were standardized at different levels depending on the chosen representation. Wherever practical, all entities that could be enumerated (e.g., units of measure, country codes) were mapped to controlled vocabularies. At the end of this process, data were fully integrated at a scientific level for data mining. Diseases were mapped onto a disease taxonomy derived from the Medical Dictionary for Regulatory Activities (MedDRA). Protein sequences were directly mapped to assays in all cases where the protein could be unambiguously identified.

Data access. To be of practical use, a data repository needs to be easily accessible. This requirement is at odds with the principles of data normalization⁴⁷ and flexibility of representation. It is therefore customary to separate the data warehouse (and data-loading activities) from access layers (data retrieval). The latter are usually data marts, sets of database tables where data are regrouped in a different way, optimized to answer specific questions. The advantage of having data marts is that queries are prepackaged and therefore faster. The drawback is that data must be copied from the data store to the data marts. This causes additional issues such as disk space shortage and scheduling of data updates and downtime. We believe that data marts are the right solution where the most common queries are already known and routinely performed. Because our database system was still highly experimental, and the number of questions we wanted to ask very high, we designed an alternative approach. We built a set of components (using Scitegic's (San Diego, CA, USA) Pipeline Pilot 4.5; <http://www.scitegic.com/>) to query, manipulate and filter the data. The lower-level components could be combined, and results from a query could be refined and/or fed into subsequent queries, generating sets of hit lists. This approach offered two advantages. The first was to perform an experimental benchmarking of the database, to find where data marts would be mostly needed to improve performance, and how the most commonly asked questions could be identified, grouped together and packaged. The second was to offer a great flexibility in interrogating the database, allowing us to cross-link the different entities in every possible way. We found that the performance drawback was acceptable for a system at this stage of maturity, mostly used for statistical analysis and post-processing, rather than for fast online data retrieval.

Preparation and analysis of chemical structures. In the study described here, all chemical structures were standardized using DayCart 4.82. A further processing step, to remove inconsistencies and identify salts and mixtures, was performed using a Pipeline Pilot protocol written in house. For the purpose of this study, all salts were stripped off the structures and the canonical tautomer of each resulting structure was identified using a standard Pipeline Pilot component. The resulting desalted canonical tautomers were loaded onto the database and used for structure matching. Molecular properties were either stored or calculated on the fly using standard Pipeline Pilot components.

Analysis of biological activity results. N-point results (e.g., IC_{50} , EC_{50} , K_i and K_d) were collected for all the molecular targets that we mapped to gene sequences. Biological assays related to more than one gene (where the particular

target could not be identified or where more than one target was involved) were kept separate. The analysis here refers to the cases where a given assay was related to a single gene. The active compounds were selected among the compounds where the best resulting activity (combining all the N-point measurement types) was found to be $<10 \mu M$. This is our definition of active compounds throughout the paper. Outliers in the biological activity results were identified with a simple automated protocol based on calculating the average distance:

$$D_i = \sqrt{(\sum_j D_{ij}^2)} \quad (1)$$

$$D_{ij} = (\log_{10}(\text{value}(i)) - \log_{10}(\text{value}(j))) \quad (2)$$

of each result in a set from all other results and flagging the ones where $D_i - D_{\min} > 1$. Here the symbols i and j refer to results from different assay experiments for the same compound and target. $D_{\min}$ is the minimum distance among all pairs of these results. The flagged sets were then manually checked and the outliers removed from the analysis.

Polypharmacology interaction network. The strength of polypharmacology interactions (P_{ij}) between two targets i and j was calculated, for all active compounds in the database, as follows:

$$P_{ij} = N_{ij} / N_{ij}^{\text{tested}} \quad (3)$$

where N_{ij}^{tested} is the number of compounds commonly tested against target i and j . N_{ij} is the number of compounds observed to bind to both targets i and j below the compound promiscuity threshold; a compound is considered shared between targets i and j if there is less than an n log difference in potency (where $n = 1$ is a tenfold difference in potency, $n = 2$ is a 100-fold difference in potency, $n = 3$ is a 1,000-fold difference in potency).

$$\log_{10}(\text{activity}_{(i)}) - \log_{10}(\text{activity}_{(j)}) \leq n \quad (4)$$

Each log order difference in potency represents a binding energy difference of $\Delta\Delta G = -1.4$ kcal/mol. We used Cytoscape⁴⁸ (<http://www.cytoscape.org/>) to display the interaction network in Figure 1 for $n = 3$. The cumulative effect of polypharmacology interactions between different targets of the same or different gene families is represented by the elements of the summarized matrix in Figure 2, calculated as

$$G_{ii} = \sum_{j \in k, j \neq i} P_{ij} \quad (5)$$

Only the cells for which enough statistics were available ($N_{ij}^{\text{tested}} > 10$) were included in the summation. A potency-difference window of $n = 1$ was used.

Figure 2 was produced using Spotfire (Somerville, MA, USA) Decision Site 7.2 (<http://www.spotfire.com/>).

Calculation of promiscuity indices. We evaluated the promiscuity of a target T in three different ways (P_1 , P_2 and P_3).

The first index was defined as follows:

$$P_1(T) = P_T < P_C >_{cc(N_{\text{actives}}(T))} \quad (6)$$

with

$$P_T = \frac{N_{\text{totalshared}}(T)}{N_{\text{actives}}(T)} \quad (7)$$

where $N_{\text{actives}}(T)$ is the number of active compounds of target T and $N_{\text{totalshared}}(T)$ is the number of active compounds of target T for which the compound promiscuity index (P_C) > 1 . (P_C of a compound (C) is defined as the total number of targets that the compound is active against.)

ANALYSIS

The second index is

$$P_2(T) = \sum_j I_{Tj} \quad (8)$$

where I_{Tj} is a matrix identical to the polypharmacology matrix, with all the values where P_{ij} is nonzero substituted by ones.

The third index was calculated by summing along rows of the matrix itself as

$$P_3(T) = \sum_j P_{Tj} \quad (9)$$

Bayesian model building. Compounds were filtered to remove structures with MW > 1,000 Da and those that failed structural quality filters (e.g., toxicophores, aggregation inhibitors, reactive groups). After the filtering, there were 617,694 experimental activities from 238,655 compounds covering 698 targets. Protein targets with fewer than ten biologically active compounds after filtering were also removed from the data set. Compound structures were transformed into FCFP_6 functional-class fingerprints. Data preparation, Bayesian analysis and model building were implemented using the Scitegic Pipeline Pilot Laplacian-corrected Bayesian classifier^{23,24} algorithm. This implementation of Bayesian statistics uses information from both the active and inactive compounds from the training set and removes features from the model, which are deemed not to be important.

Linear discriminant analysis. The biologically active compounds were filtered by chemical quality criteria to remove aggregation inhibitors and compounds with potentially reactive groups. Compounds active against more than one gene family were also removed. Of the remaining compounds, 184,687 were selected as a training set for the linear discriminant analysis (as implemented in R⁴⁹) to classify the gene family activity on a test set of 41,823 compounds. The classification was based on the following calculated molecular properties: MW, number of hydrogen-bond acceptors, number of hydrogen-bond donors, number of rotatable bonds, molecular surface area, molecular polar surface area, number of ionizable centers, clogP, Andrews' binding energy⁵⁰ and predicted molecular solubility.

Distances in reduced chemical space. We have prepared a set of 617 US Food and Drug Administration-approved oral drugs and calculated their MW, clogP, number of hydrogen-bond acceptors (H-acc), number of hydrogen-bond donors (H-don), using standard and in-house Pipeline Pilot components. In these components the H-acc atoms are defined as heteroatoms (oxygen, nitrogen, sulfur or phosphorus) with one or more lone pairs, excluding atoms with positive formal charges, amide and pyrrole-type nitrogens and aromatic oxygen and sulfur atoms in heterocyclic rings. H-don atoms are defined as heteroatoms (oxygen, nitrogen, sulfur or phosphorus) with one or more attached hydrogen atoms. These four properties are used to characterize the set in reduced chemical space. We have collected all the targets which either have potent active compounds (below 100nM) or are drug targets, and calculated the centroid [MW_T, clogP_T, H-acc_T, H-don_T] for each of these targets. The distance in reduced chemical space is defined for each target T as

$$D_T^2 = \frac{1}{4} [(1-f_{MW}(MW_T))^2 + (1-f_{clogP}(clogP_T))^2 + (1-f_{H-acc}(H-acc_T))^2 + (1-f_{H-don}(H-don_T))^2] \quad (10)$$

where the function f_k , $k = \{MW, clogP, H-don, H-acc\}$ represents the distribution of values of the molecular properties of oral drugs, normalized so that $f_k \in [0,1]$.

Note: Supplementary information is available on the Nature Biotechnology website.

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AUTHOR CONTRIBUTIONS

G.V.P., database design and production and knowledge discovery; R.H.B.S., database design and production; W.P.v.H., predictive modeling; J.S.M., chemical representation; A.L.H., database design and knowledge discovery.

COMPETING INTERESTS STATEMENT

The authors declare competing financial interests (see the *Nature Biotechnology* website for details).

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REVIEWS

BCL-2 family antagonists for cancer therapy

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Abstract | Overexpression of members of the BCL-2 family of pro-survival proteins is commonly associated with unfavourable pathogenesis in cancer. The convergence of cytotoxic stress signals on the extended BCL-2 protein family provides the biological rationale for directly targeting this family to induce apoptotic cell death. Recently, several compounds have been described that inhibit the interaction between BCL-2 family members and their natural ligand, a helical peptide sequence known as the BH3 domain. Here, we review preclinical and clinical data on these compounds, and recommend four criteria that define antagonists of the BCL-2 protein family.

Mitochondrial pathway to programmed cell death

This pathway is activated by intracellular stresses and developmental cues. Also known as the intrinsic or stress pathway, it is regulated by the extended BCL-2 protein family. By contrast, the extrinsic apoptotic pathway is activated by cell-surface death receptors.

Caspase cascade

A proteolytic cascade that is initiated by both the mitochondrial pathway and the extrinsic pathway.

The discovery that B-cell lymphoma 2 (BCL-2), a protein overexpressed in many types of cancer cells, promotes cell survival but not cell proliferation¹ has led to the view that impaired apoptosis is a critical step in tumour development². The programmed removal of unwanted, damaged or compromised cells by apoptosis is essential during embryogenesis and in maintaining cellular homeostasis. Apoptosis is also important for combating infectious microorganisms and in executing cues from cytotoxic agents used in chemotherapy. Insensitivity of tumour cells to radiation or chemotherapy can often be traced to defects in the apoptosis machinery or in the signalling pathways that would activate it in normal cells. The elucidation of these pathways over the past two decades has raised the possibility of developing therapies that induce apoptosis directly, and therefore do not rely on the upstream signals that are so frequently dysfunctional in transformed cells³. Targeting the apoptosis machinery could be useful either in removing unwanted cells, as in therapy for cancer or inflammatory disease, or in promoting cell survival, as in therapy for degenerative diseases. Our focus here is on strategies to promote cell death by antagonizing the pro-survival function of the BCL-2 protein family.

The extended BCL-2 family

The central cast of players in the mitochondrial pathway to programmed cell death is the extended BCL-2 family of proteins (FIG. 1). In mammalian cells, five pro-survival proteins — BCL-2, BCL-X_L, BCL-w, MCL1 and A1 — antagonize the pro-apoptotic function of BAK and BAX⁴. The killing activity of BAX and BAK is localized on the mitochondrial outer membrane, which becomes

permeabilized in response to death signals. As a result, cytochrome *c* is released into the cytosol, leading to the activation of the caspase cascade and the induction of apoptotic cell death.

The five pro-survival proteins and BAK/BAX all share four domains of sequence homology⁵ known as BCL-2 homology 1 (BH1), BH2, BH3 and BH4. They also have a C-terminal membrane-anchoring sequence and a similar three-dimensional structure⁶. The amino-acid sequence signature for life and death that distinguishes these two functional subfamilies remains elusive. Other key players that orchestrate apoptosis are the pro-apoptotic BH3-only proteins — so-called because they lack the BH1, BH2 and BH4 domains. Eight BH3-only proteins are known in mammals — BIM, BID, PUMA, NOXA, BAD, BME, HRK and BIK — and they are upregulated by transcription or post-translational processing in response to various stress signals⁶.

Interactions between pro-survival and pro-apoptotic proteins govern cell fate. The BH3 domain of pro-apoptotic proteins is the primary mediator of interaction with pro-survival family members. The three-dimensional structure of BCL-X_L alone⁷ and in complex with a peptide from the BAK BH3 domain⁸ (FIG. 2) laid the foundation for many subsequent mechanistic studies that led to current models for the life/death switch. In one model, a pro-survival protein, BCL-X_L for example, antagonizes BAK or BAX by binding to their BH3 domain, and a BH3-only protein relieves this antagonism by similarly binding to BCL-X_L (REF. 9). Alternatively, some data support a direct interaction between certain BH3-only proteins and BAX^{10,11}. However, in either case the structural and functional studies strongly support the idea that a

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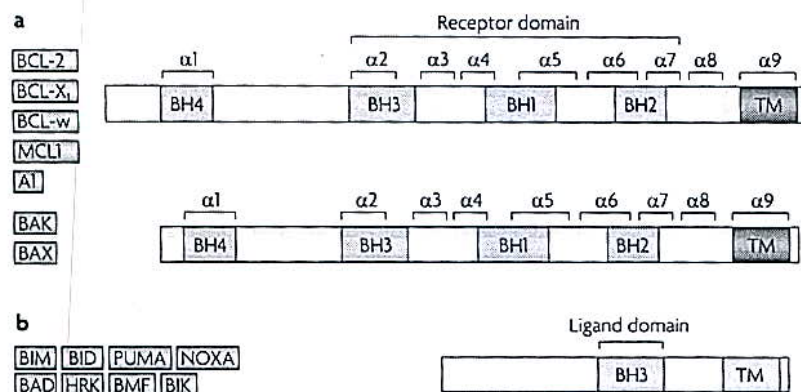


Figure 1 | The extended BCL-2 protein family. This family comprises pro-survival proteins (highlighted in green boxes) and pro-apoptotic proteins (highlighted in red boxes). Apart from this functional subdivision of the family, it is also instructive to consider a sequence/structural subdivision into multidomain proteins and BH3-only proteins. **a** | Family members sharing four BCL-2 homology (BH) domains are the multidomain proteins. These proteins share a common three-dimensional fold (FIG. 2). BCL-2-antagonist/killer (BAK) and BCL-2-associated X protein (BAX) are essential for apoptosis through the mitochondrial pathway^{12,13}. They function by permeabilizing the mitochondrial outer membrane. A third pro-apoptotic multidomain protein, BCL-2-related ovarian killer (BOK), is not yet well characterized. Pro-survival proteins are antagonists of BAK and BAX, in part by directly binding to them. **b** | Family members displaying only the BH3 domain are the BH3-only proteins. BH3-only proteins are usually unstructured prior to binding pro-survival proteins¹¹⁰. Some, but not all, are membrane anchored⁴. BH3-interacting domain death agonist (BID) is exceptional, displaying the same general fold as BCL-2 even though it lacks other BH domain signatures¹¹¹. BH3-only proteins are sensors of cellular stress and are the natural antagonists of pro-survival proteins. The BH3 domain of a BH3-only protein inserts into a binding groove on the pro-survival protein (FIG. 2); it is this interaction that the compounds reviewed in this article seek to mimic. A1, also known as BFL1/BCL2A1; BCL-w, also known as BCL2L2; BCL-X_L, also known as BCL2L1; BAD, BCL-2 antagonist of cell death; BIK, BCL-2-interacting killer; BIM, also known as BCL2L11; BMF, BCL-2-modifying factor; MCL1, myeloid cell leukaemia sequence 1; PUMA, also known as BBC3; NOXA, also known as PMAIP1; TM, transmembrane.

BH3 mimetic that binds pro-survival proteins should trigger apoptosis (FIG. 3). Cells in which both BAK and BAX have been deleted are essentially resistant to apoptosis^{12,13}, which underscores the central role of these two proteins. The use of BAX/BAK double-knockout cells in cell-death assays provides a means for deciding whether or not cytotoxic agents, including the various molecules that we review here, are killing cells by apoptosis or by non-apoptotic cell death mechanisms such as necrosis.

BCL-2 has also been linked to non-apoptotic cell death¹⁴. Both BCL-2 and BCL-X_L can bind the tumour suppressor beclin 1 (BECN1), which also contains a BH3 domain¹⁵. This interaction has been shown to inhibit autophagy. Release of this interaction by competition with a BH3-only protein (or a small-molecule mimetic) has been found to stimulate autophagy¹⁶. The role of autophagy in disease pathogenesis has only recently begun to be appreciated¹⁷, and although its role in tumour suppression has been suggested¹⁸, it is unclear whether this is due to its promotion of cell survival or cell death. Further studies with BCL-2 antagonists are needed to establish their role in triggering autophagic cell death.

Autophagy

A cellular stress response in which non-vital cell components are broken down to provide nutrients for vital processes. Autophagy may also serve as an alternative non-apoptotic mechanism for cell death under certain conditions.

Lymphopaenia

A condition marked by lower than normal circulating lymphocytes.

Thrombocytopaenia

A decrease in platelet count, defined in humans as less than 150 million per ml, which results in the potential for increased bleeding.

Functions of individual family members

The induction of apoptosis in different types of tissue and in response to different stress stimuli is a complex process that is controlled by the different BCL-2 family members. For example, knockout studies have demonstrated that BCL-X_L is essential in embryogenesis for survival of erythroid progenitors and neuronal cells¹⁹, and that BCL-2 is required for survival of mature T and B lymphocytes²⁰. In addition, it has been shown that MCL1 is required for implantation and for survival of haematopoietic stem cells and progenitor B and T lymphoid cells²¹, and that BCL-w sustains developing sperm cells²². Furthermore, BCL-X_L is essential for regulating platelet survival²³. So, the consequences of blocking the function of individual pro-survival proteins are target-specific; antagonists of BCL-2 might be expected to cause lymphopaenia and antagonists of BCL-X_L might be expected to cause thrombocytopaenia.

Selective interactions of the BH3-only proteins with pro-survival proteins are now well documented²⁴. BIM and PUMA are promiscuous in that they both bind to all five pro-survival proteins, whereas BAD and NOXA have complementary binding profiles. The basis for the selectivity of BAD and NOXA is becoming clear as mutated BH3 sequences with non-natural selectivity patterns are discovered (FIG. 4). Furthermore, different stress signals activate different BH3-only proteins. For example, NOXA²⁵ and PUMA²⁶ are triggered by the p53 pathway. By contrast, BIM, in addition to its major role in haematopoietic homeostasis²⁷, is the primary responder to endoplasmic reticulum stress²⁸.

High expression levels of BCL-2 were first associated with follicular lymphoma. Indeed, BCL-2 overexpression has since been reported in many tumour types and is often correlated with poor survival²⁹. Similarly, MCL1 (REF. 30) is overexpressed in various haematopoietic and lymphoid cancers, including multiple myeloma³¹ and chronic lymphocytic leukaemia³². Furthermore, levels of BCL-X_L expression in the 60 cell lines in the National Cancer Institute's anticancer drug screen (NCI-ACDS) panel are highly correlated with resistance to 122 standard chemotherapeutic agents³³.

To initiate cell death in an otherwise healthy cell, either BAK or BAX must be relieved of its suppression by the relevant pro-survival proteins. BCL-X_L and MCL1 seem to be the key direct antagonists of BAK³⁴, whereas control of BAX includes BCL-2 (REF. 9). So, for BAK-mediated cell death an antagonist of both BCL-X_L and MCL1 is required. Failing that, a direct agonist of the killing activity of BAK (or BAX) would suffice. Apart from reports that the BH3-only proteins BIM and BID are direct activators of BAX^{10,11}, no progress has yet been reported in finding such molecules.

To kill tumour cells, the particular expression profile of pro-survival and BH3-only protein expression is likely to determine the required specificity of an antagonist^{10,24}. A central issue will be to establish whether or not a therapeutic window exists in which the expected on-target toxicities on normal tissues can be managed while maintaining efficacious dosing. A summary of the putative antagonists reviewed in this article can be found in TABLE 1.

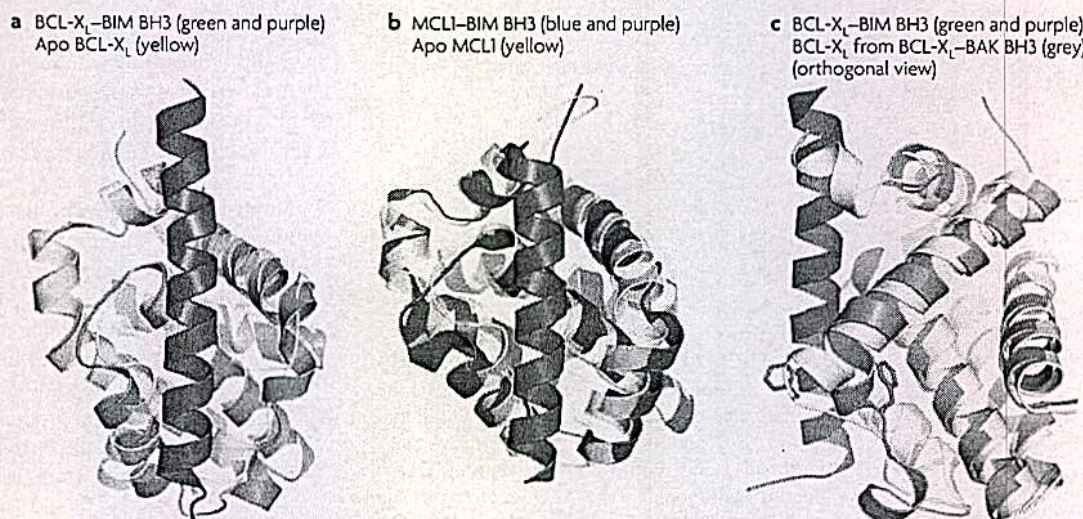


Figure 2 | Structures of pro-survival proteins with BH3 ligands. Crystal or solution structures are known for all five pro-survival family members, together with various complexes with BCL-2 homology 3 (BH3) ligands^{112–115}. The common fold for the multidomain BCL-2 family members is an eight-helix bundle⁸ that creates a groove into which helical BH3-domain peptides bind⁸. Among the amino acids that form the binding groove are elements of the BH1, BH2 and BH3 domains. Binding of BH3-domain peptides is associated with different degrees of conformational reorganization: large in the case of BCL-X_L binding to BIM BH3 (REF. 116) (a), and smaller in the case of MCL1 binding to BIM BH3 (REFS 115, 117) (b). Furthermore, different BH3 sequences may bind to markedly different conformations of the target as seen in a comparison of the binding of BCL-X_L to the BH3 domains of BAK⁸ and BIM¹¹⁶, and as highlighted by the different positions of F105 (shown in magenta) in these structures (c). This extreme plasticity in the target is not conducive to ligand design. Not surprisingly, the progress reported here in discovering organic BCL-2 antagonists largely derives from screening chemical libraries. Protein Data Bank (PDB)¹¹⁸ entries for the displayed structures: BCL-X_L-BIM BH3 is 1PQ1; Apo BCL-X_L is 1PQ0; MCL1-BIM BH3 is 2NL9; Apo MCL1 is 1WSX; and BCL-X_L-BAK BH3 is 1BXL.

It is important to remember that the five pro-survival proteins function on intracellular membranes where they are tail-anchored, and that essentially all of the discovery and structural studies have been carried out with protein constructs lacking the C-terminal membrane anchor. Nevertheless, a good correlation is observed between the binding potency of antagonists (peptides or small molecules) to these truncated proteins and their cellular killing activity.

Peptides and peptide mimetics

The binding profiles of the eight BH3-only proteins and their structures in complex with their targets (FIGS 4,5) provide a starting point for designing antagonists²⁴. In the original study showing that NOXA is selective for MCL1 and A1, a designed mutant form of the NOXA BH3 sequence was described, which can also bind to BCL-X_L and BCL-w²⁴. Subsequently, other non-natural BH3 sequences with novel binding profiles have been described, including a mutant of BIM that is selective for MCL1 (REF. 35). This mutant (BIM2A) was discovered by phage display and its sequence lacks both the critical leucine residue (the canonical hydrophobic residue h2; see FIG. 4) and a large hydrophobic residue at position h4. These phage studies have emphasized just how diverse the BH3-binding grooves of the different pro-survival molecules are, indicating that target-selective small-molecule antagonists might be found.

Modified peptides. Several short peptides representing the BH3 domain of BH3-only proteins have been designed. The lack of binding affinity of these peptides is probably due in part to their lack of helicity³⁶, and various attempts at reinforcing the helical structure of such peptides have been published. A recent approach³⁷ relies on “stapling”, whereby two non-essential amino acids of the helical BH3 sequence are replaced by non-natural amino acids bearing a side chain with a double-bond terminus, which is connectable via a metathesis reaction. Such stapling enhances the helicity of 23-mer BH3 peptides from BID and BIM. One use of these stapled peptides has been to further investigate direct activation of BAX by BIM and BID³⁸. Although peptides rarely progress to late-stage clinical trials because of poor pharmacological properties, the stapled peptides demonstrate cell-membrane permeability and extended half-life in the presence of serum in comparison with the non-constrained peptides³⁷, which offers the possibility of *in vivo* efficacy. These dual properties seem to be a consequence of the helix staple both shielding the peptide from proteases and affording membrane permeability. The cell-based activity observed on leukaemic cell lines translates into reduced leukaemia progression and an extended median time of death in animal models (11 days versus 5 days for untreated animals).

Another approach to stabilizing peptide-like structures is the use of α/β peptides, which, in one case, had

Endoplasmic reticulum stress

Cellular disturbances causing an accumulation of unfolded proteins in the endoplasmic reticulum, which results in activation of the unfolded protein response.

Metathesis reaction

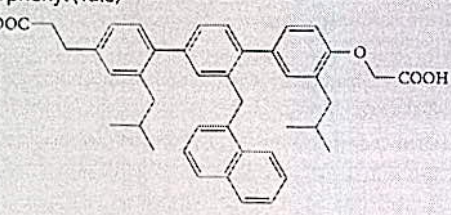
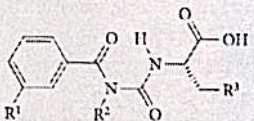
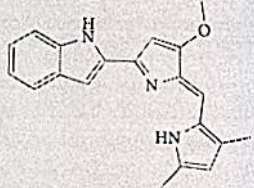
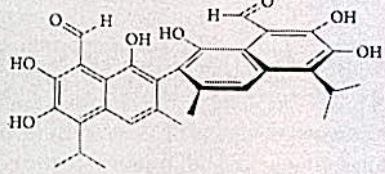
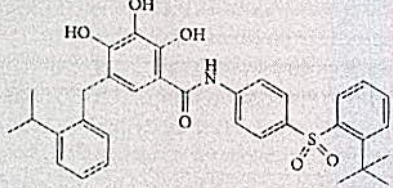
A transition metal-catalysed reaction used to manipulate alkenes.

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acute myeloid lymphoma and myelofibrosis (NCI's clinical trial results; see Further information). Combination treatments involve the following drugs: bortezomib, rituximab, carboplatin and etoposide. It was shown that multiple day infusions are well tolerated with manageable

adverse events^{64–66}. Notably, in one trial, a patient suffering from acute myeloid leukaemia achieved complete haematological recovery, which was sustained over an 8-month treatment regime of once-weekly infusions of GX15-070 (REF. 64).

Table 1 | Putative BCL-2 inhibitors (part 1)

Molecule (company/institution)	Binding data*	Cellular activity†	Successful combinations‡	Additional notes
SAHB _A (Aileron) ^{37,38} 	• K_d for BCL-2 = 39 nM • EC_{50} for BAX (FL) = 885 nM • EC_{50} for BAX = 293 nM • EC_{50} for BCL-X_L (FL) = 230 nM • EC_{50} for BCL-X_L = 46 nM	pre-B ALL, AML	Not known	• Has activity in animal models • Status: preclinical
Terphenyl (Yale) ⁴⁰ 	• K_i for BCL-X_L = 114 nM	Not known	No	• Has no activity in animal models • Status: discovery
Benzoylureas (WEHI) ⁴³ 	• IC_{50} for BCL-X_L = 1–20 μM	Not known	No	• Has no activity in animal models • Status: discovery
GX15-070 (GeminX) ^{55–65} 	• IC_{50} for BCL-X_L = 4.7 μM • IC_{50} for BCL-2 = 1.1 μM • IC_{50} for BCL-w = 7.0 μM • IC_{50} for MCL1 = 2.9 μM	LC, BC, CC, PC, CoC, CLL, AML, MCL	Bortezomib, rituximab, cisplatin, vinorelbine, Ara-C, U0126, lapatinib, gefitinib, docetaxel, topotecan, fludarabine, ABT-737	• Has activity in animal models • Administered intravenously • Status: Phase I/II
AT-101 (–) gossypol (Ascenta) ^{71–76,79} 	• IC_{50} for BCL-X_L = 480 nM • IC_{50} for BCL-2 = 320 nM • IC_{50} for MCL1 = 180 nM	DLBCL, CLL, MM	Rituximab, cyclophosphamide, topotecan, docetaxel, prednisone, temezolomide	• Has activity in animal models • Orally available • Status: Phase I/II
TW37 (UMCCC) ^{78,80} 	• K_i for BCL-X_L = 1.11 μM • K_i for BCL-2 = 0.29 μM • K_i for MCL1 = 0.26 μM	PC, HM	U0126	• Has activity in animal models • Status: discovery

* IC_{50} and EC_{50} values are as reported from competition studies with BH3 peptides. K_i values are as calculated from IC_{50} values in fluorescent polarization assays. K_d values are as reported from direct binding experiments. All studies were performed on membrane anchorless versions of pro-survival proteins except where indicated as full length (FL). †As a single agent or in combination in susceptible cell types. ‡In cell and animal models. ALL, acute lymphoblastic leukaemia; AML, acute myeloid leukaemia; BC, breast cancer; CC, cervical cancer; CLL, chronic lymphocytic leukaemia; CoC, colon cancer; DLBCL, diffuse large B cell lymphoma; FL, follicular lymphoma; HM, human melanoma; HML, human myeloid leukaemia; HL, human lymphoma; HPL, human premyelocytic leukaemia; LC, lung cancer; MCL, mantle cell lymphoma; MM, multiple myeloma; MZL, marginal zone lymphoma; NHL, non-Hodgkin's lymphoma; PC, prostate cancer; R-CHOP, rituximab, cyclophosphamide, doxorubicin, vincristine and prednisolone; UMCCC, University of Michigan Comprehensive Cancer Center, Ann Arbor, USA; WEHI, The Walter and Eliza Hall Institute of Medical Research, Victoria, Australia.

Three recent reports call into question the proposed mechanism of action of GX15-070. First, the compound kills BAK/BAX double-knockout MEFs as potently ($EC_{50} \sim 5 \mu M$) as it kills wild-type cells⁶⁷, which suggests that non-apoptotic cell death is occurring. Second, GX15-070 also causes cell-cycle arrest (S-G2); these differential apoptotic and clonogenic activities on acute myeloid leukaemia cell lines — with IC_{50} values of $4 \mu M$ and $0.1 \mu M$, respectively⁶² — lead to the conclusion that “Bcl-2 independent targets of this agent may have clinical applicability”. Third, no correlation was observed between sensitivity of non-small-cell lung cancer cells to the drug and expression levels of BCL-2 family members⁵⁹. Despite the data supporting a role for GX15-070 as a BCL-2 family antagonist, it remains to be shown that this is the source of its antitumour activity in animal models⁵⁸.

AT-101. The natural product gossypol, derived from the cotton plant, has been used for a long time in a wide range of therapeutic contexts⁶⁸. Its link to apoptosis is more recent, highlighted by the discovery that the (–) enantiomer of this atropoisomeric molecule bears most of the antiproliferative properties observed in the racemate⁶⁹. Further studies have revealed that this particular enantiomer binds BCL-2 ($K_i = 320$ nM), BCL- X_L ($K_i = 480$ nM) and MCL1 ($K_i = 180$ nM)⁷⁰. The compound is being developed by Ascenta as AT-101. In animal models, AT-101 has shown single-agent activity in cell lines of multiple myeloma (median effect between $5 \mu M$ and $10 \mu M$); in B-cell lymphomas⁷¹; and in cell samples from patients with primary chronic lymphocytic leukaemia (50% reduction in cell viability at $2 \mu M$)^{72,73}. Therefore, the cytotoxic potency of AT-101 is similar to that of GX15-070 despite an approximately fivefold advantage in binding potency to BCL-2 targets. Synergy with rituximab has also been demonstrated⁷³.

The good pharmacokinetic properties of this molecule and its low toxicity have allowed progression in to clinical trials⁷⁴. In a Phase I open-label trial with patients with previously untreated chronic lymphocytic leukaemia, AT-101 was accompanied with mostly manageable adverse effects (gastrointestinal toxicities) that were reversible upon cessation of the treatment⁷⁴. Efficacy was also recorded with reduced lymphadenopathy (eight out of eight) and splenic enlargement (six out of six). In Phase II open-label trials in combination with rituximab, two dosing regimens were applied with the aim of reducing the adverse effects. A pulsed administration with a higher dose (80 mg per day versus 30 mg per day in the case of continuous daily dosing) of AT-101 was found to be the most effective at maintaining good exposure as well as reducing side effects⁷⁵. With this regimen, a preliminary report documented a partial response in three out of six patients, while the three remaining patients were still under treatment. The response rate in the cohort subjected to the continuous treatment in association with rituximab was 38%, with four out of eight patients showing significant reduction in leukaemic cell count and splenomegaly⁷⁶.

Gossypol can kill MEFs in a BAK/BAX-independent manner^{51,77}, which suggests that its cytotoxic activity is not solely related to its capacity to bind to pro-survival proteins. The same observation led to the conclusion that the compound transformed BCL-2 into a pro-apoptotic molecule⁷⁷. Similar experiments are needed with the active enantiomer AT-101.

Gossypol derivatives. Based on modelling of the binding interaction between AT-101 and BCL-2, several studies have investigated analogues of these molecules for improved properties. Three compounds have emerged to date: TW37 (REF. 78), TM-1206 (REF. 70) and another acylpyrrogallol derivative⁷⁹. Submicromolar affinities for BCL-2 and MCL1 have been achieved using fluorescent polarization and ELISA assays. For example, for TW37 and TM-1206, IC_{50} values of 290 nM and 110 nM, respectively, were achieved for BCL-2, and IC_{50} values of 260 nM and 150 nM, respectively, were achieved for MCL1. These effects are reported to translate into cell-based activity. MDA-MB-231 and PC3 cell lines respond to treatment with the compounds; TW37 has an IC_{50} of 200 nM in PC3 cell lines⁷⁸. TW37 has also been shown to synergize with the MAPK/ERK kinase (MEK) inhibitor U0126 (REF. 80), highlighting the connection between the MAPK pathway and the BCL-2 protein family. Notably, subapoptotic levels of TW37 (50 nM) are sufficient to induce a cytostatic effect on endothelial cells, which indicates that other biological pathways participate in the antiproliferative activity of this compound⁸¹.

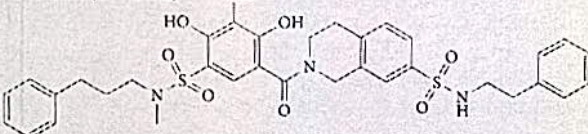
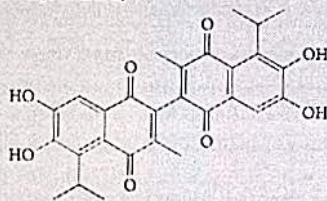
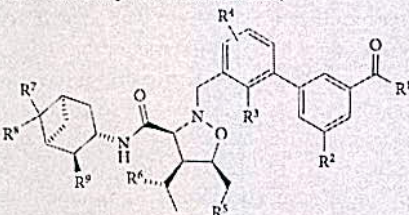
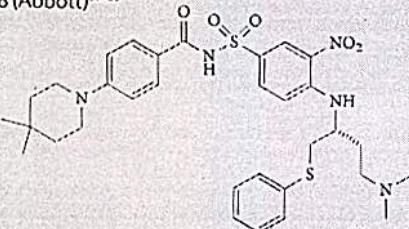
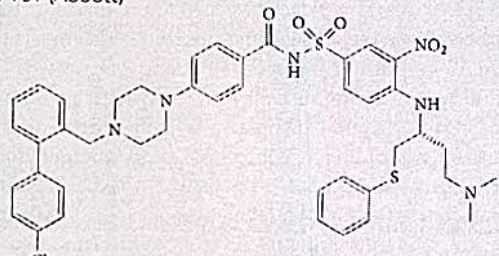
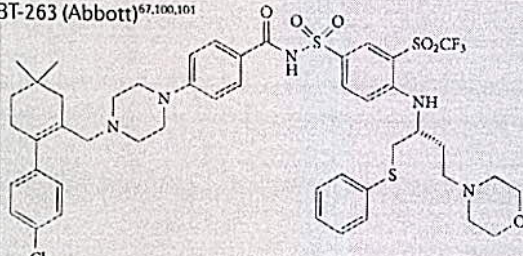
Apogossypolone is another gossypol derivative that has submicromolar binding affinity for BCL-2, MCL1 and BCL- X_L (K_i values of 35 nM, 25 nM and 660 nM, respectively)⁸². Apogossypolone inhibited the growth of follicular lymphoma cell lines (WSU-FSCCL, IC_{50} of 109 nM). On primary tumour cell lines, apogossypolone had IC_{50} values of $0.7 \mu M$ (mantle cell lymphoma sample); $1.75 \mu M$ (marginal zone lymphoma sample); and 1.3 – $3.5 \mu M$ (chronic lymphocytic leukaemia samples). In xenograft mice injected intraperitoneally with WSU-FSCCL tumour cell lines, apogossypolone treatment (25 mg per kg either intravenously or intraperitoneally) resulted in extended survival times and protected against tumour-cell infiltration into the bone marrow⁸².

The cytotoxicity of these various gossypol derivatives needs to be determined in BAK/BAX double-knockout cells to establish their mechanisms of action.

Isoxazolidine derivatives. Based on the diversity-oriented synthesis approach developed by Schreiber and co-workers⁸³, a series of isoxazolidine-based small molecules were found to interact with BCL-2 and BCL- X_L . A large number of elaborated small molecules around this structural family have been described⁸⁴, with apparent selectivity towards BCL-2 (the best compounds exhibiting binding affinities below 1 nM). This series of compounds led to a collaboration between Infinity Pharmaceuticals and Novartis in January 2005, and several compounds are currently in preclinical evaluation.

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Table 1 | Putative BCL-2 inhibitors (part 2)

Molecule (company/institution)	Binding data*	Cellular activity [†]	Successful combinations [‡]	Additional notes
TM-1206 (UMCCC) ⁷⁹ 	• IC₅₀ for BCL-X_L = 0.64 μM • IC₅₀ for BCL-2 = 0.11 μM • IC₅₀ for MCL1 = 0.15 μM	BC	No	• Has no activity in animal models • Status: discovery
Apogossypolone (UMCCC) ⁸² 	• K_i for BCL-X_L = 660 nM • K_i for BCL-2 = 35 nM • K_i for MCL1 = 25 nM	NHL, pre-B ALL, MCL, CLL, MZL	No	• Has activity in animal models • Status: discovery
Isooxazolidine (Infinity Pharmaceuticals) ⁸⁴ 	• IC₅₀ for BCL-X_L < 1 μM • IC₅₀ for BCL-2 < 1 nM	Not known	Not known	• Status: preclinical
A-385358 (Abbott) ⁸⁷⁻⁸⁹ 	• K_i for BCL-X_L = 0.8 nM • K_i for BCL-2 = 67 nM	LC, ALL	Paclitaxel, etoposide, doxorubicin, cisplatin	• Has activity in animal models • Status: preclinical
ABT-737 (Abbott) ^{51,67,85,89,95-98} 	• K_i for BCL-X_L < 0.5 nM • K_i for BCL-2 < 1 nM • K_i for BCL-w = 0.9 nM • K_i for MCL1 > 1 μM	AML, LC, NHL, HL, MM, BC, AML, CC, MCL	Etoposide, PD98059, roscovitine, R-roscovitine, bortezomib, carboplatin, melphalan, dexamethasone, doxorubicin, Ara-C, rituximab, imatinib	• Has activity in animal models • No oral availability • Status: preclinical
ABT-263 (Abbott) ^{67,100,101} 	• K_i for BCL-X_L < 0.5 nM • K_i for BCL-2 < 1 nM • K_i for BCL-w < 1 nM • K_i for MCL1 = 550 nM	AML, NHL, CLL, MM, ALL, LC, MCL	Rituximab, R-CHOP, bortezomib	• Has activity in animal models • Orally available • Status: Phase I

*IC₅₀ and EC₅₀ values are as reported from competition studies with BH3 peptides. K_i values are as calculated from IC₅₀ values in fluorescent polarization assays. K_d values are as reported from direct binding experiments. All studies were performed on membrane anchorless versions of pro-survival proteins except where indicated as full length (FL). †As a single agent or in combination in susceptible cell types. ‡In cell and animal models. ALL, acute lymphoblastic leukaemia; AML, acute myeloid leukaemia; BC, breast cancer; CC, cervical cancer; CLL, chronic lymphocytic leukaemia; CoC, colon cancer; DLBCL, diffuse large B cell lymphoma; FL, follicular lymphoma; HM, human melanoma; HML, human myeloid leukaemia; HL, human lymphoma; HPL, human premyelocytic leukaemia; LC, lung cancer; MCL, mantle cell lymphoma; MM, multiple myeloma; MZL, marginal zone lymphoma; NHL, non-Hodgkin's lymphoma; PC, prostate cancer; R-CHOP, rituximab, cyclophosphamide, doxorubicin, vincristine and prednisolone; UMCCC, University of Michigan Comprehensive Cancer Center, Ann Arbor, USA; WEHI, The Walter and Eliza Hall Institute of Medical Research, Victoria, Australia.

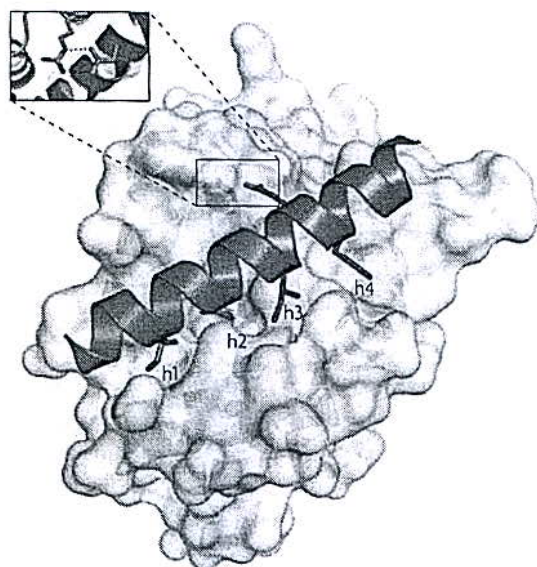


Figure 5 | Interactions between pro-survival BCL-2 proteins and BH3 ligands as exemplified by the MCL1-BIM BH3 complex. Four conserved hydrophobic residues on the BH3 domain (h1–h4) project into four hydrophobic pockets within the ligand binding groove. In addition, a conserved aspartic acid on the BH3 domain forms a salt bridge to a conserved arginine on the pro-survival protein (inset). Crystal structure obtained from Protein Data Bank entry 2NL9 (REF. 115).

ABT-737 and ABT-263. Using the structure–activity relationship (SAR) by nuclear magnetic resonance (NMR) fragment screening approach, ligands with high binding efficiency for the hydrophobic groove of BCL- X_L have been discovered^{85,86}. Multiple iterations of medicinal chemistry and NMR structure analysis, including design strategies to reduce serum binding⁸⁷, led to the discovery of compounds with BCL- X_L and BCL-2 inhibitory activity. A-385358 (compound 73R) has K_i values of 0.8 nM and 67 nM, respectively, against these targets. It also shows cell killing activity in transformed FL5.12 cells overexpressing BCL- X_L (with an EC_{50} value of 0.47 μ M)^{87,88}. Only moderate single-agent activity was recorded on tumour cell lines, and cytotoxicity was further compromised in the presence of serum. A-385358 synergizes with multiple cytotoxic agents (such as etoposide, doxorubicin, cisplatin and paclitaxel) *in vitro*, and with paclitaxel *in vivo*⁸⁸. Further studies led to the discovery of ABT-737, a molecule with high affinity for BCL-2 and BCL- X_L (IC_{50} values of <1 nM and <0.5 nM, respectively)^{85,89}. Notably, the binding affinity is maintained even in 10% human serum (IC_{50} of 35 nM for BCL- X_L).

Unlike all other discovery programmes described to date, this drive to high affinity has been informed by solution structures of complexes, from the earliest fragment hits to close relatives of ABT-737 (for example, compounds 43a and 43b⁸⁹). A crystal structure of the BCL- X_L complex with ABT-737 — the first published crystal structure of an organic ligand complexed with a BCL-2 family protein⁹⁰ — further highlights the

plasticity of the BCL- X_L protein. It reveals a deepening of the p2 pocket, beyond what is seen in the complex with the BIM BH3 peptide, to accommodate the terminal chlorophenyl moiety (FIG. 6). Furthermore, the acidic acylsulphonamide does not interact with R139 from the BH1 domain of BCL- X_L as expected from the earlier fragment studies, but instead is in close proximity to the N terminus of the helix α 5. This interaction in the bound state does not preclude potentially essential interactions between the acylsulphonamide and R139 during association.

With regards to molecular mass, number of heteroatoms, functional groups and rotatable bonds, ABT-737 substantially fails the classical rules of drug-likeness^{91,92}. It is not orally available, but has an acceptable pharmacokinetic profile when administered intraperitoneally, which allows animal studies to be conducted. It is proving to be a useful tool molecule in apoptosis research⁹³. Further modifications have resulted in a clinical candidate, ABT-263, which retains potency for BCL-2, BCL- X_L and BCL-w, and has moderate oral bioavailability in animal models ($F = 20\%$)⁶⁷. Oral absorption of ABT-263 is limited by its dissolution. Its half-life averages 4.6–8.4 hours (in mouse, rat, dog and monkey)⁶⁷. C_{max} following oral dosing in severe combined immunodeficiency mice affords appropriate levels of the drug to observe strong therapeutic effects⁶⁷. These observations have translated to other animal species and are now under scrutiny in humans during the current Phase I clinical trials in which ABT-263 is delivered orally.

The binding specificity of ABT-737 (and ABT-263) is reminiscent of the BH3-only protein BAD (FIG. 4). Accordingly, ABT-737 has limited activity in wild-type MEFs but is very potent in MEFs expressing NOXA or in cells lacking MCL1 (REFS 51,94). This dependency on low MCL1 levels for efficacy has been found in a large panel of cell types including acute myeloid leukaemia cells⁹⁵, small-cell lung cancer cells^{96,97}, and FDC-P1, MCF7 and HeLa carcinoma cells⁵¹. Synergy with agents such as etoposide and carboplatin is probably due to their capacity to indirectly control MCL1 by upregulation of PUMA, NOXA and BIM⁹⁶. Sensitivity to ABT-737 can be restored through downregulation of MCL1 by silencing with small interfering RNA^{94,95,97}, by interleukin 3 (IL3) withdrawal^{51,67}, by cyclin-dependent kinase inhibition with seliciclib and by protein synthesis inhibition with cycloheximide^{51,94}.

ABT-737 and ABT-263 have both been found to be active as a single agent in haematological malignancies; for example, chronic lymphocytic leukaemia⁹⁸, multiple myeloma⁹⁹, leukaemia cell lines such as MV-4-11, RS4;11 and CEM/C1, lymphoma cell lines such as Granta-519 and in acute lymphoblastic leukaemia^{67,100}. ABT-737 and ABT-263 are also active as a single agent in small-cell lung carcinoma solid tumour cell lines; for example, NCI-H146, NCI-H889 and NCI-H1963 (REFS 67,101). These responsive cell lines all have low levels of MCL1 (REFS 67,96,98,100,101). Positive results in cell-based assays translated into efficacy in *in vivo* experiments using xenografts with similar tumour cell lines. Significant reduction of tumour volume has been

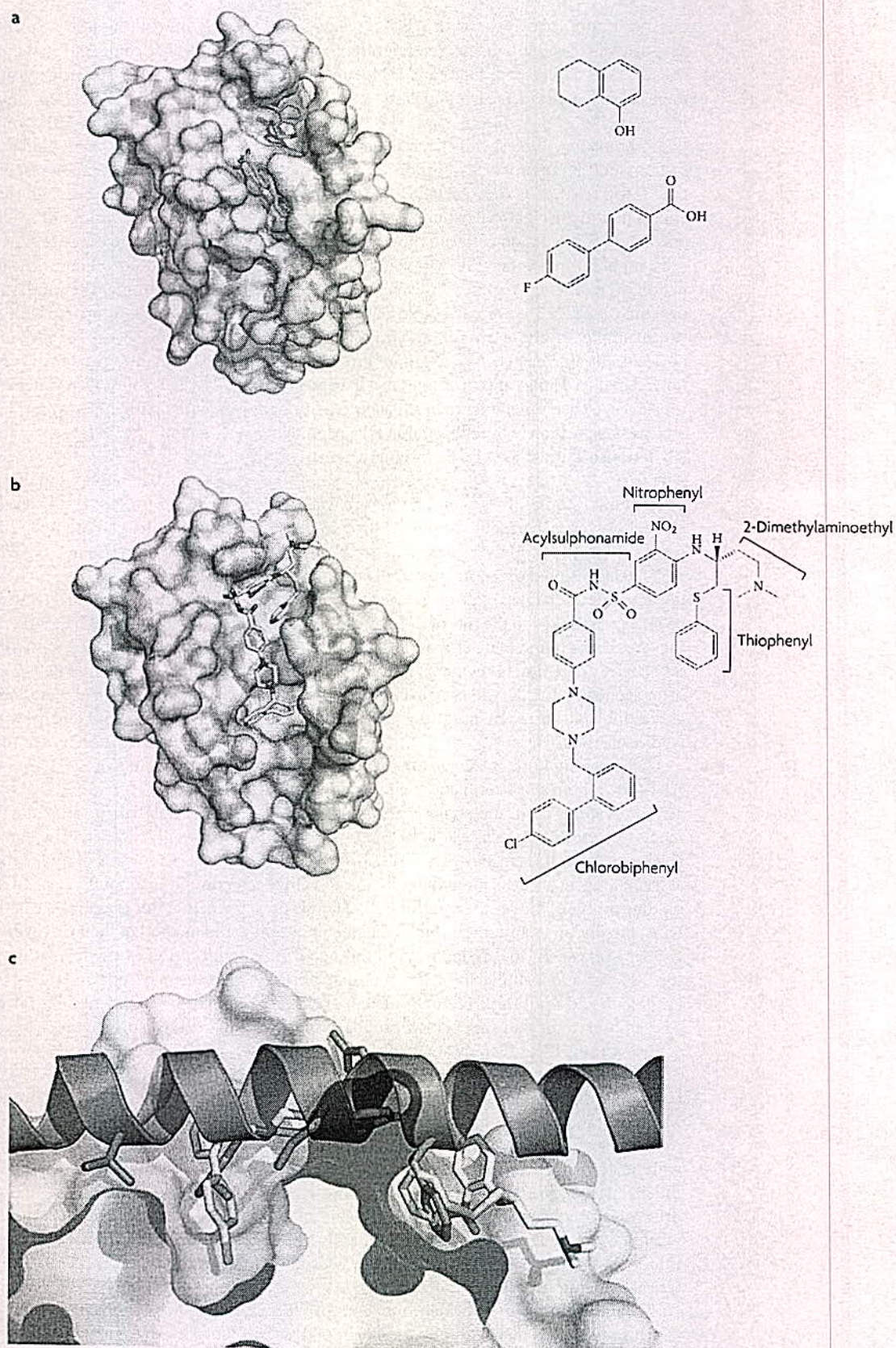


Figure 6 | Structural studies related to the discovery of ABT-737. **a** | Structure of BCL-X_L bound to fragments discovered by nuclear magnetic resonance (Protein Data Bank (PDB)¹¹⁸ entry 1YSG). **b** | BCL-X_L bound to ABT-737 (PDB entry 2YXJ). **c** | Overlay of BCL-X_L-ABT-737 complex with BCL-X_L-BIM BH3 complex (PDB entry 1PQ1). For clarity BCL-X_L from the BCL-X_L-BIM BH3 structure is not displayed in the overlay.

observed upon treatment with ABT-263 as a single agent in small-cell lung cancer xenografts (complete response was observed for H889 and H146) and haematological xenografts (acute lymphoblastic leukaemia and RS4;11)^{67,95,98}.

Combination studies with classical cytotoxic agents or targeted therapeutics have produced encouraging preclinical results. *In vivo*, ABT-263 had a significant effect on diffuse large B-cell lymphoma cell tumour progression in combination with rituximab, and on mantle cell lymphoma in combination with R-CHOP (a combination chemotherapy regimen)⁶⁷. Even in tumour types in which ABT-263 is weakly active as a single agent (for example, multiple myeloma), combination with bortezomib can enhance *in vivo* tumour growth inhibition⁶⁷. Small-cell lung cancer cell lines that have acquired resistance to chemotherapies such as paclitaxel, through increased expression of the efflux pump P-glycoprotein 1 (PGP1; also known as ABCB1), remain sensitive to ABT-263 (REF. 101).

In animal studies, these compounds are well tolerated. However, a sharp but temporary drop in platelet count is observed following exposure to ABT-737 or ABT-263. This phenomenon is clinically distinct from the most common types of drug-induced thrombocytopaenia and is linked to the role of BCL-X_L in controlling the circulating blood platelet lifespan^{23,102,103}. No effect on platelet progenitors is evident. This on-target effect of antagonizing BCL-X_L will need to be considered when establishing dosing regimens during the current Phase I studies of ABT-263.

Cells lacking both BAX and BAK are resistant to ABT-737 in both short-term and long-term clonogenic assays⁵¹. A strong correlation is observed in many experiments (see above) between cellular BCL-2 family protein levels and the cell's sensitivity to ABT-737 or ABT-263 as a single agent, a correlation that is in accord with the binding profile of the compounds for BCL-2 family proteins. Finally, an enantiomer of the related compound A-385358 has a 20-fold reduction in binding affinity for BCL-X_L, and a similar trend in cytotoxic potency (18-fold, 7-fold and 14-fold in three different cell lines)⁶⁸. These observations all support the proposed mode of action as a true BCL-2 family antagonist.

Conclusions

Many anticancer drugs ultimately activate the apoptotic machinery via the BH3-only proteins¹⁰⁴. So, a cell treated with imatinib or paclitaxel will probably exhibit most of the characteristics of apoptotic death^{105,106}; however, these drugs are not antagonists of the BCL-2 protein family. So what makes a molecule a true functional BCL-2 family inhibitor? Setting aside possible effects on autophagy (see above), we suggest four criteria:

- Biological activity must be dependent on BAK/BAX. This is a requirement for induction of apoptosis by BH3-only proteins, and is therefore a requirement for cell killing by a functional BH3 mimetic.
- The binding affinity of the compound to at least one BCL-2 family pro-survival protein must be in the low nanomolar (or tighter) range. Defining this boundary

is difficult, because the pharmacological properties of the drug come into play and could allow compounds with high systemic exposure and high C_{max} to compensate for weaker binding affinity. However, most of the endogenous BH3-only proteins or the peptides derived from them bind their pro-survival counterparts with low nanomolar affinity²⁴. Conversely, peptides that have been altered and that exhibit weaker binding affinities fail to elicit biological activity¹⁰⁷. Some of the data we have reviewed here support a threshold value for the affinity to be near 1 nM. For example, the moderate cell-based activity of A-385358, compared with ABT-737, has been attributed to its weak (67 nM) affinity for BCL-2 (REF. 89). The enantiomer of ABT-263 displays a K_i value for BCL-X_L of 3–17 nM depending on the assay, compared with <0.5 nM for ABT-263 itself, and yet the enantiomer is largely inactive in cell-based killing assays⁶⁷.

- Cytotoxic activity should be correlated with cellular BCL-2 protein family levels and the binding profile of the drug to the BCL-2 family members. MCL1-mediated resistance to ABT-737 is an example of this criterion.
- Treatment of animals with a BCL-2 antagonist should trigger relevant biomarkers, such as acute reduction in platelet levels for a BCL-X_L antagonist, and reduced lymphocytes for a BCL-2 antagonist. Other markers for antagonism of these targets, not evident from gene knockouts, may emerge with new tool compounds. It remains to be seen what the markers might be for an MCL1 antagonist.

Further data on all of the compounds reviewed here are keenly awaited. The compounds from Abbott are outliers in terms of molecular mass, but there are not enough examples yet of BCL-2 antagonists to know whether this is a correlate of their binding potency or of the method of their discovery, fragment-based screens. Inhibition of the formation of protein–protein complexes may require larger-than-usual molecules, and their development does raise special issues¹⁰⁸. Several NMR structural studies have accompanied the discovery of ABT-263, but no experimental structures of complexes of AT-101 or GX15-070 with their targets have been reported so far, despite their progression into clinical studies. Acquiring such data should be a priority as it may lead to more potent compounds.

The progress described here in antagonizing the BCL-2 proteins validates the target for cancer indications. The degree of diversity found in compounds binding to a specific target site is evident enough even when that site is the small and relatively rigid active centre of an enzyme such as influenza neuraminidase¹⁰⁹. The protein target we describe here is both large and flexible, and as such is expected to be able to host a truly diverse array of ligands. It will be fascinating to observe future progress in targeting the site. Meanwhile, further clinical development of the current compounds is keenly awaited, as is the response of tumours to challenge by this novel class of cytotoxic agents.

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Competing interests statement

The authors declare competing financial interests: see web version for details.

DATABASES

UniProtKB: <http://ca.expasy.org/sprot>
BAD|BAK|BAX|BBC3|BCL-2|BCL2A1|BCL2L1|BCL2L2|BCL2L11|BID|BIM|BME|BOK|BCL1|PMAIP1

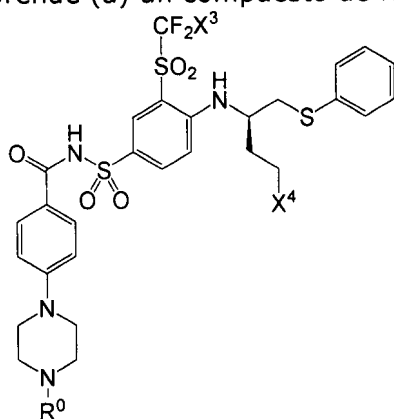
FURTHER INFORMATION

NCI's clinical trial results:
www.cancer.gov/search/ResultsClinicalTrialsAdvanced.aspx?protocolsearchid=4748252

ALL LINKS ARE ACTIVE IN THE ONLINE PDF

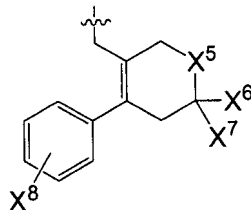
REIVINDICACIONES:

1. Una composición farmacéutica para suministrar en forma oral
CARACTERIZADA PORQUE comprende (a) un compuesto de fórmula I:



donde X^3 es cloro o flúor; y

- (1) X^4 es azepan-1-ilo, morfolin-4-ilo, 1,4-oxazepan-4-ilo, pirrolidin-1-ilo, $N(CH_3)_2$, $N(CH_3)(CH(CH_3)_2)$, 7-azabicyclo[2.2.1]heptan-1-ilo o 2-oxa-5-azabicyclo[2.2.1]hept-5-ilo; y R^0 es



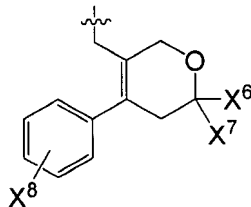
donde

X^5 es O, CH_2 , $C(CH_3)_2$ o CH_2CH_2 ;

X^6 y X^7 son ambos hidrógeno o ambos metilo; y

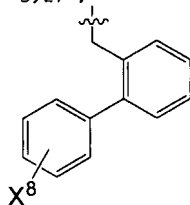
X^8 es flúor, cloro, bromo o yodo; o

- (2) X^4 es azepan-1-ilo, morfolin-4-ilo, pirrolidin-1-ilo, $N(CH_3)(CH(CH_3)_2)$ o 7-azabicyclo[2.2.1]heptan-1-ilo; y R^0 es



donde X^6 , X^7 y X^8 son según se dijo anteriormente; o

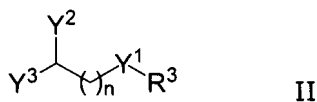
(3) X^4 es morfolin-4-ilo o $N(CH_3)_2$; y R^0 es



donde X^8 es según se dijo anteriormente;

o una sal farmacéuticamente aceptable de la misma;

(b) un antioxidante calcógeno más pesado farmacéuticamente aceptable que comprende uno o más compuestos antioxidantes de Fórmula II:



donde

n es 0, 1 o 2;

Y^1 es S o Se;

Y^2 es NHR^1 , OH o H, donde R^1 es alquilo o alquilcarbonilo;

Y^3 es $COOR^2$ o CH_2OH , donde R^2 es H o alquilo; y

R^3 es H o alquilo;

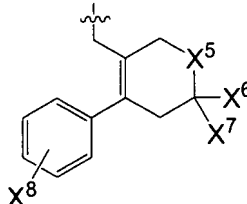
donde los grupos alquilo opcionalmente están sustituidos de manera independiente con uno o más sustituyentes que se seleccionan independientemente entre el grupo que consiste en carboxilo, alquilcarbonilo, alcóxicarbonilo, amino y alquilcarbonilamino; donde cada grupo alquilo o grupo alcóxi comprende entre 1 y 18 átomos de carbono; una sal farmacéuticamente aceptable de los mismos; o, donde Y^1 es S y R^3 es H, un dímero $-S-S-$ de los mismos o una sal farmacéuticamente aceptable de dicho dímero; y (c) un vehículo lípido farmacéuticamente aceptable que contiene menos del 5% en peso de agua; donde dicho compuesto y el antioxidante están formando una solución en el vehículo lípido.

2. La composición de la reivindicación 1, CARACTERIZADA PORQUE, en el

compuesto de fórmula I, X^3 es fluoro.

3. La composición de la reivindicación 1 o la reivindicación 2, CARACTERIZADA PORQUE, en el compuesto de fórmula I, X^4 es morfolin-4-ilo.

4. La composición de cualquiera de las reivindicaciones 1-3, CARACTERIZADA PORQUE, en el compuesto de fórmula I, R^0 es



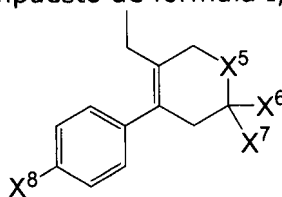
donde

X^5 es O, CH_2 , $C(CH_3)_2$ o CH_2CH_2 ;

X^6 y X^7 son ambos hidrógeno o ambos metilo; y

X^8 es flúor, cloro, bromo o yodo.

5. La composición de cualquiera de las reivindicaciones 1-3, CARACTERIZADA PORQUE, en el compuesto de fórmula I, R^0 es



donde

X^5 es O, CH_2 , $C(CH_3)_2$ o CH_2CH_2 ;

X^6 y X^7 son ambos hidrógeno o ambos metilo; y

X^8 es flúor, cloro, bromo o yodo.

6. La composición de la reivindicación 5, CARACTERIZADA PORQUE, en el compuesto de fórmula I, X^5 es CH_2 o $C(CH_3)_2$ y/o cada uno de X^6 y X^7 es metilo y/o X^8 es cloro.

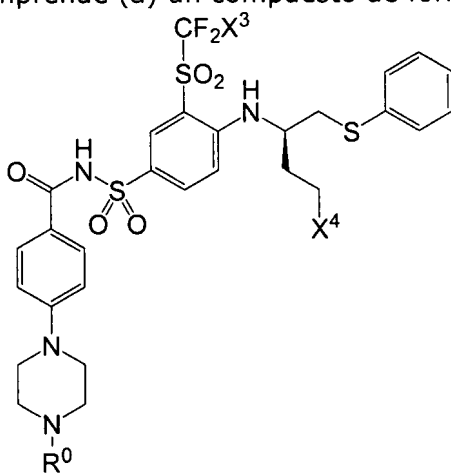
7. La composición de la reivindicación 1, CARACTERIZADA PORQUE el compuesto de fórmula I es ABT-263 (N-(4-(4-((2-(4-clorofenil)-5,5-dimetil-1-ciclohex-

1-en-1-il)metil)piperazin-1-il)benzoil)-4-(((1R)-3-(morfolin-4-il)-1-((fenilsulfanil)metil)propil)amino-3-((trifluorometil) sulfonil)bencenosulfonamida) o una sal de la misma.

8. La composición de la reivindicación 7, CARACTERIZADA PORQUE el compuesto es la base libre de ABT-263 o ABT-263 *di*-HCl.

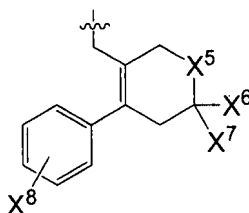
9. La composición de la reivindicación 7 o la reivindicación 8, CARACTERIZADA PORQUE el compuesto está presente en una cantidad de entre 10 y 500 mg/ml como equivalente de base libre.

10. Una composición farmacéutica para suministrar en forma oral CARACTERIZADA PORQUE comprende (a) un compuesto de fórmula I:



donde X^3 es cloro o flúor; y

(1) X^4 es azepan-1-ilo, morfolin-4-ilo, 1,4-oxazepan-4-ilo, pirrolidin-1-ilo, $N(CH_3)_2$, $N(CH_3)(CH(CH_3)_2)$, 7-azabicyclo[2.2.1]heptan-1-ilo o 2-oxa-5-azabicyclo[2.2.1]hept-5-ilo; y R^0 es



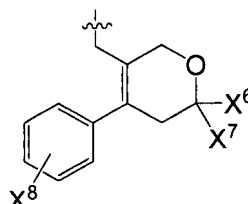
donde

X^5 es O, CH_2 , $C(CH_3)_2$ o CH_2CH_2 ;

X^6 y X^7 son ambos hidrógeno o ambos metilo; y

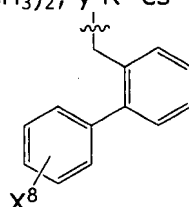
X^8 es flúor, cloro, bromo o yodo; o

(2) X^4 es azepan-1-ilo, morfolin-4-ilo, pirrolidin-1-ilo, $N(CH_3)(CH(CH_3)_2)$ o 7-azabicyclo[2.2.1]heptan-1-ilo; y R^0 es



donde X^6 , X^7 y X^8 son según se dijo anteriormente; o

(3) X^4 es morfolin-4-ilo o $N(CH_3)_2$; y R^0 es



donde X^8 es según se dijo anteriormente;

o una sal farmacéuticamente aceptable de la misma;

(b) un antioxidante calcógeno más pesado farmacéuticamente aceptable donde el antioxidante comprende uno o más compuestos que se seleccionan entre el grupo que consiste en N-acetilcisteína, éster butílico de N-acetilcisteína, éster dodecílico de N-acetilcisteína, éster etílico de N-acetilcisteína, éster metílico de N-acetilcisteína, éster octílico de N-acetilcisteína, éster propílico de N-acetilcisteína, éster estearílico de N-acetilcisteína, éster tetradecílico de N-acetilcisteína, éster tridecílico de N-acetilcisteína, N-acetilmetionina, éster butílico de N-acetilmetionina, éster dodecílico de N-acetilmetionina, éster etílico de N-acetilmetionina, éster metílico de N-acetilmetionina, éster octílico de N-acetilmetionina, éster propílico de N-acetilmetionina, éster estearílico de N-acetilmetionina, éster tetradecílico de N-acetilmetionina, éster tridecílico de N-acetilmetionina, N-acetilselenocisteína, éster butílico de N-acetilselenocisteína,

éster dodecílico de N-acetilselenocisteína, éster etílico de N-acetilselenocisteína, éster metílico de N-acetilselenocisteína, éster octílico de N-acetilselenocisteína, éster propílico de N-acetilselenocisteína, éster estearílico de N-acetilselenocisteína, éster tetradecílico de N-acetilselenocisteína, éster tridecílico de N-acetilselenocisteína, N-acetilselenometionina, éster butílico de N-acetilselenometionina, éster dodecílico de N-acetilselenometionina, éster etílico de N-acetilselenometionina, éster metílico de N-acetilselenometionina, éster octílico de N-acetilselenometionina, éster propílico de N-acetilselenometionina, éster estearílico de N-acetilselenometionina, éster tetradecílico de N-acetilselenometionina, éster tridecílico de N-acetilselenometionina, cisteína, éster butílico de cisteína, éster dodecílico de cisteína, éster etílico de cisteína, éster metílico de cisteína, éster octílico de cisteína, éster propílico de cisteína, éster estearílico de cisteína, éster tetradecílico de cisteína, éster tridecílico de cisteína, cistina, éster dibutílico de cistina, éster di(dodecílico) de cistina, éster dietílico de cistina, éster dimetílico de cistina, éster dioctílico de cistina, éster dipropílico de cistina, éster diestearílico de cistina, éster di(tetradecílico) de cistina, éster di(tridecílico) de cistina, N,N-diacetilcistina, N,N-diacetilcistina éster dibutílico de, éster dietílico de N,N-diacetilcistina, éster di(dodecílico) de N,N-diacetilcistina, éster dimetílico de N,N-diacetilcistina, éster dioctílico de N,N-diacetilcistina, éster dipropílico de N,N-diacetilcistina, éster diestearílico de N,N-diacetilcistina, éster di(tetradecílico) de N,N-diacetilcistina, éster di(tridecílico) de N,N-diacetilcistina, tiodiglicolato de dibutilo, tiodipropionato de dibutilo, tiodiglicolato de di(dodecilo), tiodipropionato de di(dodecilo), tiodiglicolato de dietilo, tiodipropionato de dietilo, tiodiglicolato de dimetilo, tiodipropionato de dimetilo, tiodiglicolato de dioctilo, tiodipropionato de dioctilo, tiodiglicolato de dipropilo, tiodipropionato de dipropilo, tiodiglicolato de diestearilo, tiodipropionato de

diestearilo, tiodiglicolato de di(tetradecilo), tiodipropionato de di(tetradecilo), homocisteína, éster butílico de homocisteína, éster dodecílico de homocisteína, éster etílico de homocisteína, éster metílico de homocisteína, éster octílico de homocisteína, éster propílico de homocisteína, éster estearílico de homocisteína, éster tetradecílico de homocisteína, éster tridecílico de homocisteína, metionina, éster butílico de metionina, éster dodecílico de metionina, éster etílico de metionina, éster metílico de metionina, éster octílico de metionina, éster propílico de metionina, éster estearílico de metionina, éster tetradecílico de metionina, éster tridecílico de metionina, S-metilcisteína, éster butílico de S-metilcisteína, éster dodecílico de S-metilcisteína, éster etílico de S-metilcisteína, éster metílico de S-metilcisteína, éster octílico de S-metilcisteína, éster propílico de S-metilcisteína, éster estearílico de S-metilcisteína, éster tetradecílico de S-metilcisteína, éster tridecílico de S-metilcisteína, selenocisteína, éster butílico de selenocisteína, éster dodecílico de selenocisteína, éster etílico de selenocisteína, éster metílico de selenocisteína, éster octílico de selenocisteína, éster propílico de selenocisteína, éster estearílico de selenocisteína, éster tetradecílico de selenocisteína, éster tridecílico de selenocisteína, selenometionina, éster butílico de selenometionina, éster dodecílico de selenometionina, éster etílico de selenometionina, éster metílico de selenometionina, éster octílico de selenometionina, éster propílico de selenometionina, éster estearílico de selenometionina, éster tetradecílico de selenometionina, éster tridecílico de selenometionina, ácido tiodiglicólico, ácido tiodipropiónico, tioglicerol, y sales de los mismos; y

(c) un vehículo lípido farmacéuticamente aceptable que contiene menos del 5% en peso de agua;

donde dicho compuesto y el antioxidante están formando una solución en el vehículo lípido.

11. La composición de cualquiera de las reivindicaciones 1-9 o 10, CARACTERIZADA PORQUE el antioxidante está presente en una proporción molar con el compuesto de fórmula I de entre 1:10 y 2:1.

12. La composición de cualquiera de las reivindicaciones 1-9 o 10, CARACTERIZADA PORQUE el antioxidante está presente en una proporción molar con el compuesto de fórmula I de entre 8:10 y 10:8.

13. La composición de cualquiera de las reivindicaciones 1-9 o 10-12, CARACTERIZADA PORQUE el vehículo comprende un fosfolípido y un componente solubilizante.

14. La composición de la reivindicación 13, CARACTERIZADA PORQUE el fosfolípido comprende fosfatidilcolina.

15. La composición de la reivindicación 13 o la reivindicación 14, CARACTERIZADA PORQUE el componente solubilizante del vehículo comprende uno o más glicoles, glicólidos y/o materiales glicéridos.

16. La composición de la reivindicación 13 o la reivindicación 14, CARACTERIZADA PORQUE el agente solubilizante comprende uno o más triglicéridos de cadena media.

17. La composición de la reivindicación 16, CARACTERIZADA PORQUE el agente solubilizante también comprende uno o más mono- y/o diglicéridos de cadena media.

18. La composición de cualquiera de las reivindicaciones 13-17, CARACTERIZADA PORQUE además comprende un tensioactivo no fosfolípido.

19. La composición de la reivindicación 18, CARACTERIZADA PORQUE el tensioactivo no fosfolípido comprende uno o más Polysorbates.

20. La composición de la reivindicación 19, CARACTERIZADA PORQUE los uno o más Polysorbates tiene un valor de peróxido menor de 5.

21. La composición de cualquiera de las reivindicaciones 1-9,

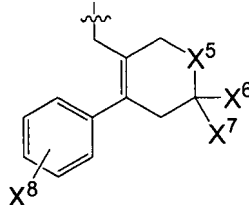
CARACTERIZADA PORQUE el antioxidante es poco soluble en lípidos y la composición comprende agua en una cantidad de hasta 1% en peso, suficiente para introducir el antioxidante como una solución madre acuosa.

22. La composición de la reivindicación 21, CARACTERIZADA PORQUE el antioxidante poco soluble en lípidos está presente en una cantidad de entre 0,02% y 0,2% en peso de la composición.

23. La composición de la reivindicación 10, CARACTERIZADA PORQUE, en el compuesto de fórmula I, X^3 es fluoro.

24. La composición de la reivindicación 23, CARACTERIZADA PORQUE, en el compuesto de fórmula I, X^4 es morfolin-4-ilo.

25. La composición de cualquiera de las reivindicaciones 23-24, CARACTERIZADA PORQUE, en el compuesto de fórmula I, R^0 es



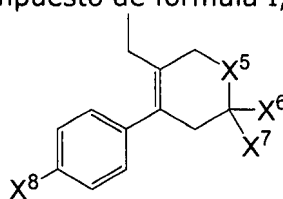
donde

X^5 es O, CH_2 , $C(CH_3)_2$ o CH_2CH_2 ;

X^6 y X^7 son ambos hidrógeno o ambos metilo; y

X^8 es flúor, cloro, bromo o yodo.

26. La composición de cualquiera de las reivindicaciones 23-24, CARACTERIZADA PORQUE, en el compuesto de fórmula I, R^0 es



donde

X^5 es O, CH_2 , $C(CH_3)_2$ o CH_2CH_2 ;

X⁶ y X⁷ son ambos hidrógeno o ambos metilo; y

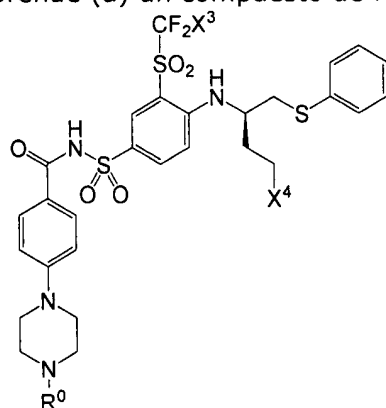
X⁸ es flúor, cloro, bromo o yodo.

27. La composición de la reivindicación 10, CARACTERIZADA PORQUE el compuesto de fórmula I es ABT-263 (N-(4-(4-(((2-(4-clorofenil)-5,5-dimetil-1-ciclohex-1-en-1-il)metil)piperazin-1-il)benzoil)-4-(((1R)-3-(morfolin-4-il)-1-((fenilsulfanil)metil)propil)amino-3-((trifluorometil) sulfonil)bencenosulfonamida) o una sal de la misma.

28. La composición de la reivindicación 27, CARACTERIZADA PORQUE el compuesto es la base libre de ABT-263 o ABT-263 *di*-HCl.

29. La composición de la reivindicación 27 o la reivindicación 28, CARACTERIZADA PORQUE el compuesto está presente en una cantidad de entre 10 y 500 mg/ml como equivalente de base libre.

30. Una composición farmacéutica para suministrar en forma oral CARACTERIZADA PORQUE comprende (a) un compuesto de fórmula I:

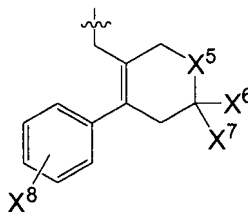


I

donde X³ es cloro o flúor; y

(1) X⁴ es azepan-1-ilo, morfolin-4-ilo, 1,4-oxazepan-4-ilo, pirrolidin-1-ilo, N(CH₃)₂, N(CH₃)(CH(CH₃)₂), 7-azabicyclo[2.2.1]heptan-1-ilo o 2-oxa-5-azabicyclo[2.2.1]hept-5-ilo; y R⁰ es

120



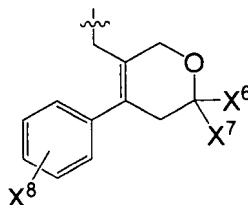
donde

X^5 es O, CH_2 , $C(CH_3)_2$ o CH_2CH_2 ;

X^6 y X^7 son ambos hidrógeno o ambos metilo; y

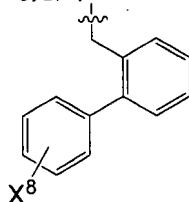
X^8 es flúor, cloro, bromo o yodo; o

(2) X^4 es azepan-1-ilo, morfolin-4-ilo, pirrolidin-1-ilo, $N(CH_3)(CH(CH_3)_2)$ o 7-azabicyclo[2.2.1]heptan-1-ilo; y R^0 es



donde X^6 , X^7 y X^8 son según se dijo anteriormente; o

(3) X^4 es morfolin-4-ilo o $N(CH_3)_2$; y R^0 es



donde X^8 es según se dijo anteriormente;

o una sal farmacéuticamente aceptable de la misma;

(b) un antioxidante calcógeno más pesado farmacéuticamente aceptable poco soluble en lípidos que se selecciona entre el grupo que consiste en sulfitos, bisulfitos, metabisulfitos, tiosulfatos y mezclas de los mismos.

31. La composición de la reivindicación 30, CARACTERIZADA PORQUE el antioxidante poco soluble en lípidos comprende metabisulfito de sodio o potasio.

32. La composición de cualquiera de las reivindicaciones 1-9 o 10-31, CARACTERIZADA PORQUE además comprende un agente quelante farmacéuticamente

aceptable.

33. La composición de la reivindicación 32, CARACTERIZADA PORQUE el agente quelante comprende EDTA o un sal del mismo.

34. Una composición farmacéutica en forma de una solución líquida CARACTERIZADA PORQUE comprende entre 5% y 20% en peso de base libre de ABT-263, entre 15% y 60% en peso de fosfatidilcolina, entre 7% y 30% en peso de triglicéridos de cadena media, entre 7% y 30% en peso de mono- y diglicéridos de cadena media, entre 7% y 30% de tensioactivo Polysorbate 80, entre 0,02% y 0,2% en peso de metabisulfito de sodio o potasio, entre 0,003% y 0,01% de EDTA o una sal del mismo, y entre 0,2% y 0,8% de agua.

35. La composición de la reivindicación 34, CARACTERIZADA PORQUE además comprende una cápsula donde se encapsula dicha solución líquida.

36. La composición de la reivindicación 34 o la reivindicación 35, CARACTERIZADA PORQUE la solución líquida consiste esencialmente en entre 5% y 20% en peso de base libre de ABT-263, entre 15% y 60% en peso de fosfatidilcolina, entre 7% y 30% en peso de triglicéridos de cadena media, entre 7% y 30% en peso de mono- y diglicéridos de cadena media, entre 7% y 30% de tensioactivo Polysorbate 80, entre 0,02% y 0,2% en peso de metabisulfito de sodio o potasio, entre 0,003% y 0,01% de EDTA o una sal del mismo, y entre 0,2% y 0,8% de agua.

37. La composición de la reivindicación 35, CARACTERIZADA PORQUE comprende una cápsula de gelatina dura de tamaño 0 con una solución líquida encapsulada dentro de la misma que comprende 50 mg de base libre de ABT-263, 150 mg de fosfatidilcolina, 75 mg de triglicéridos de cadena media, 90 mg de mono- y diglicéridos de cadena media, 90 mg de tensioactivo Polysorbate 80, 0,25 mg de metabisulfito de sodio o potasio, 0,025 mg de EDTA o una sal del mismo, y 2,5 mg de agua.