

**CASTELLANOS & CO LTDA**  
**ABOGADOS**  
Calle 94 A No. 7 A-09  
Bogotá, D.C. – COLOMBIA

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



No. 06-108716- -00017-0000

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Act. 412 REPOSPRESRECU Folios: 54

Señor

**SUPERINTENDENTE DE INDUSTRIA Y COMERCIO**

Superintendencia de Industria y Comercio

Ministerio de Desarrollo Económico

E. S. D.

Re.: Expediente No. **06.108.716**

Solicitud de Patente, para la invención:

**"INDAZOLES, BENZOTIAZOLES, BENZOISOTIAZOLES, BENZISOXAZOLES Y  
PREPARACION Y USOS DE ESTOS"**

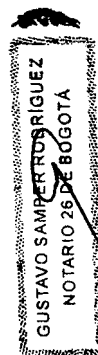
Solicitante: **MEMORY PHARMACEUTICALS CORPORATION**

### **RECURSO DE REPOSICION**

Yo, **MARGARITA CASTELLANOS ABONDANO**, mayor de edad, domiciliada e identificada como aparece al pié de mi firma, apoderada de la sociedad solicitante en el asunto de la referencia, atentamente acudo a su Despacho con el fin de interponer **RECURSO DE REPOSICIÓN** (art. 50 C.C.A.) para que se revoque en su totalidad la resolución No. **11751** del 29 de febrero de 2012, notificada por edicto desfijado el 30 de marzo de 2012.

#### **1. LA RESOLUCION No. 11751**

La Superintendencia de Industria y Comercio expidió la Resolución No. 11751 por la cual se niega la patente que fue solicitada el día 25 de octubre de 2006, para la invención denominada: **"INDAZOLES, BENZOTIAZOLES, BENZOISOTIAZOLES, BENZISOXAZOLES Y PREPARACION Y USOS DE ESTOS"**, y radicada bajo el expediente N° **06.108.716**.



## 2. FUNDAMENTO DE LA RESOLUCION No. 11751

La Resolución 11751 establece en su artículo sexto que, las reivindicaciones 1 a 13, contenidas en los folios 733 a 737, no cumplen con el requisito de nivel inventivo.

## 3. FUNDAMENTOS DEL RECURSO

Presento a su Despacho las siguientes consideraciones que justifican la revocatoria de la Resolución N° 11751 del 29 de febrero de 2012:

El artículo 45 establece:

*“Si la oficina nacional competente encontrara que la invención no es patentable o que no cumple con alguno de los requisitos establecidos en esta Decisión para la concesión de la patente, lo notificará al solicitante. Este deberá responder a la notificación dentro del plazo de sesenta días contados a partir de la fecha de la notificación. Este plazo podrá ser prorrogado por una sola vez por un período de treinta días adicionales.*

*Cuando la oficina nacional competente estimara que ello es necesario para los fines del examen de patentabilidad, podrá notificar al solicitante dos o más veces conforme al párrafo precedente.*

*Si el solicitante no respondiera a la notificación dentro del plazo señalado, o **si a pesar de la respuesta subsistieran los impedimentos para la concesión**, la oficina nacional competente denegará la patente.” (Negrita fuera de texto)*

Como bien lo establece el Artículo anterior, la Oficina Nacional denegará la patente si subsisten los impedimentos para la concesión, a pesar de haber dado respuesta al requerimiento de fondo. Respetuosamente manifiesto que éste no es el caso de la presente solicitud, toda vez que el solicitante atendió a cabalidad las observaciones de ese Despacho en cuanto a la claridad, unidad de invención y novedad, superando los “impedimentos” relativos a estos tres requisitos. Lo anterior se desprende de la resolución impugnada por la cual ese Despacho no niega el privilegio de patente por carencia de novedad, falta de claridad o de unidad de invención, sino por falta de nivel inventivo.

Pongo de presente que la altura inventiva de la solicitud no fue objetada durante el trámite de ésta solicitud y que, en consecuencia, el solicitante no tuvo la oportunidad de



presentar respuesta sobre éste requisito de patentabilidad, a partir de la cual este Despacho concluyera que el “impedimento” relativo a nivel inventivo subsistiera.

Así entonces, si ese Despacho consideró que la solicitud no cumplía con el requisito mencionado debió notificar nuevamente al solicitante y dar así la oportunidad de controvertir las objeciones y presentar pruebas a favor del nivel inventivo. No obstante, procedió a negar el privilegio de patente sobre la base de un nuevo “impedimento” que no surgió en el examen de fondo de la solicitud.

Pongo de presente que el documento D3 (JP2002-030084), citado en la resolución impugnada, ni siquiera era conocido por el solicitante como arte previo que afectara la patentabilidad de la invención. Como podrá verificar ese Despacho, en el Oficio No. 662 del 14 de enero de 2011 se citaron los documentos D1 (WO02100858), D2 (US2003073707) y D3 (WO2004029050), además como documentos relevantes para la novedad de la invención y **no** para su altura inventiva.

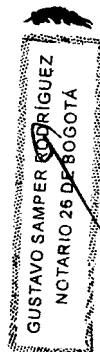
Es claro entonces que con la Resolución No. 11751 se violaron las disposiciones del Artículo 45 de la Decisión 486 sobre la facultad que tiene la Oficina Nacional para denegar una patente cuando, a pesar de la respuesta, subsistan los impedimentos para la concesión, y respetuosamente solicito se revoque la resolución.

### 3.1 Finalidad de las actuaciones administrativas

Dada la denegación de la patente para la presente invención con base en argumentos adicionales a los expuestos durante el trámite, es preciso mencionar que **la función Administrativa está concebida en favor de los administrados y para velar por la efectividad de sus derechos e intereses** (Artículo 2, Código Contencioso Administrativo). Para tal fin la Constitución y la ley han contemplado unos principios orientadores de las actuaciones de la Administración, los cuales deben ser respetados por ésta.

El artículo 3 del Código Contencioso Administrativo, Principios orientadores, establece que: *“Las actuaciones administrativas se desarrollarán con arreglo a los principios de economía, celeridad, eficacia, imparcialidad, publicidad y contradicción y, en general, conforme a las normas de esta parte primera.”*

El inciso 8 del artículo mencionado, establece:



“En virtud del principio de contradicción, los interesados tendrán oportunidad de conocer y de controvertir esas decisiones por los medios legales.”

Acudiendo a lo anterior, con la Resolución No. 11751 se vulneró el derecho de mi representada a conocer las objeciones relativas al nivel inventivo y a controvertirlas por medio de una respuesta debidamente notificada de conformidad con el Artículo 45 de la Decisión 486. Adicionalmente, teniendo en cuenta que el señor examinador citó un documento adicional (D3), resultaba necesaria la emisión de un Oficio de fondo trasladando las nuevas objeciones al solicitante.

Resulta claro entonces que con la actuación de la administración en este trámite, no se procedió en concordancia con el artículo 2º del C.C.A., ni con los principios orientadores de la función Administrativa, por cuanto con la orden de negar el privilegio de patente, habiéndose superado todas y cada una de las objeciones expuestas por el examinador en la última acción oficial de fondo No. 662, la Administración no dio la oportunidad al solicitante de presentar sus argumentos y pruebas a favor del nivel inventivo, requisito que no aparecía como afectado en dicha acción oficial. Adicionalmente, reitero que en la resolución No. 11751, se cita un documento del arte previo D3, el cual no fue mencionado en la acción oficial No. 662, y por obvias razones el solicitante no tuvo la ocasión de defender la invención ante las objeciones realizadas con base en tal documento.

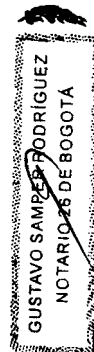
En vista de los anteriores argumentos, se debe reconocer que la negación se basó en hechos diferentes a los impugnados con ocasión del artículo 45, y solicito se revoque la resolución No. 11751 dando continuidad al trámite de la presente solicitud.

En cuanto a las objeciones sobre el nivel inventivo de la presente solicitud, apporto lo siguientes argumentos:

### **3.2 Incumplimiento del Artículo 18 de la Decisión 486**

El examinador afirma en la resolución No. 11751 que la persona del oficio normalmente versada en la materia técnica incluiría la característica, de que grupos heteroarilo bicíclicos se encuentren unidos al grupo farmacofórico carbamoil-quinuclidina por cualquier átomo de carbono (D3), a los compuestos de D1 para así llegar a los compuestos aquí reivindicados.

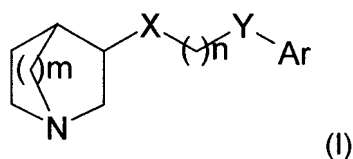
Ante dicha afirmación, advierto que para el nivel inventivo se exige que el técnico medio que realiza el examen debe partir del conocimiento general que él tiene sobre el estado de la técnica y realizar el cotejo comparativo con su apreciación de conjunto, determinando si con tales conocimientos técnicos existentes ha podido o no producirse tal invención. Sin



embargo, el análisis hecho por el examinador de patentes sobre la solicitud en cuestión falla al establecer si a partir de los conocimientos previos en la materia el experto en la técnica tendría la **motivación y las enseñanzas necesarias** para llegar de manera obvia a la presente invención.

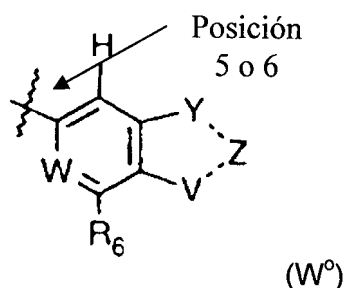
De este modo, la decisión de negar el privilegio de patente se basó en un análisis retrospectivo erróneo, donde el examinador se limitó a señalar que la presente invención puede derivarse de la combinación del arte previo, siendo que tal combinación no resulta obvia ni es sugerida en el arte previo, es decir, el examinador llegó a una conclusión de obviedad partiendo de la invención y asimilándola a lo divulgado en D1 y D3, cuando la invención no es una combinación de estos dos documentos y los mismos tampoco sugieren que al combinarse sea posible hallar los compuestos de la presente invención. En ningún momento el examinador argumentó que las diferencias específicas de los compuestos de mi representado con los del arte previo obedecieran a un propósito funcional o relacionado con algún tipo de patrón o comportamiento específico estudiado antes.

En cuanto al documento D3 (JP 2002-030084), este enseña un amplio rango de compuestos de la fórmula (I) que también son agonistas de  $\alpha 7$  nAChR:



En donde Ar corresponde a una variedad de grupos heteroarílicos bicíclicos, con un grupo puente  $-X-(CH_2)_n-Y$  entre Ar y un grupo azabicyclo que puede ser  $-NH-CO-$ . Sin embargo, el documento D3 no muestra ningún compuesto en que el grupo heteroarilo bicíclico sea indazol, tampoco revela compuestos en que se tenga un enlace puente de estructura  $-NH-CO-$  entre Ar y el azabicyclo, y ninguno de los compuestos revela un grupo puente unido por la posición 3 del heteroarilo bicíclico.

Por su parte D1 (WO 02/100858) presenta compuestos de fórmula (I) en que  $W^o$  es la primera de tres subfórmulas,



donde W es  $-CH-$  y V-Y-Z es  $-N-NH=CH-$  (páginas 2-4)

Sin embargo, cuando W<sup>o</sup> es dicha subfórmula, se tiene que solo puede estar unido al resto del compuesto desde su posición 5 o 6 dependiendo de los heteroátomos que se encuentren (página 20, líneas 3-4 y último párrafo; página 21, líneas 30-31; ejemplo 14). En comparación, en la reivindicación 1 de mi representado el grupo A está unido al resto de la estructura desde las posiciones 3, 4 o 7. Adicionalmente, D1 no enseña ningún compuesto en que el grupo indazol se encuentre sustituido por un R1 como en la presente solicitud. De este modo, aún si los compuestos de D1 se modificaran con base en los patrones de sustitución de D3, el resultado no sería un compuesto comprendido por la presente solicitud, y el hecho que los isómeros de posición 3 estén excluidos del género de compuestos presentados por D1 podría sugerirle a la persona versada en la materia técnica que no es de esperarse que estos tengan propiedades similares a las que presentan los isómeros de posiciones 5 y 6.

Adicionalmente, es claro que el arte previo no enseña cómo modificar los compuestos existentes para llegar a los novedosos compuestos de indazol y de isobenzotiazol que propone el solicitante como compuestos alternativos que actúan como ligandos para el subtipo  $\alpha 7$  nAChR.

Cabe resaltar que de acuerdo con la guía dada por el Manual Andino de Patentes (no como marco normativo pero sí como una guía válida para el examen de solicitudes de patente de invención, preparada por autoridades competentes y basada en la Decisión 486), un compuesto tiene nivel inventivo cuando este posee una estructura inesperada:

*“Es un caso poco frecuente. La estructura del nuevo compuesto no hubiera podido ser deducida por el técnico medio en la materia. El examinador no necesita examinar si este compuesto posee o no un uso o efecto sorprendente puesto que la mera estructura ya le confiere el nivel inventivo”. (Subrayado fuera de texto).*

Entonces, teniendo en cuenta que la estructura de los compuestos reivindicados es novedosa y no se deriva de manera evidente del estado de la técnica, esto es, es inventiva por sí misma, no es necesario presentar resultados comparativos que demuestren una actividad superior frente a otros compuestos que, por lo demás se apartan estructuralmente de los reivindicados. En este caso particular se debe demostrar que los compuestos nuevos e inventivos tienen la actividad aducida por el solicitante, razón por la cual adjuntó al presente recurso una tabla que reporta los datos de actividad para varios de los compuestos reivindicados.

<sup>1</sup> Manual Andino de Patentes. Nivel inventivo en áreas específicas de la tecnología. Pag 82-83

En este orden de ideas, el nivel inventivo de la presente invención se basa en el hecho de que los compuestos reivindicados resultan inesperados y completamente alternativos a los compuestos revelados por el arte previo.

A favor de los anteriores argumentos, presento un estudio comparativo (anexo 2) entre los compuestos con la estructura base de la presente solicitud y los compuestos divulgados por el documento D1, en donde se contrasta la sustitución en la **posición 3 del grupo indazol** (invención) con la posición 5 o posición 6. Puede notarse que dicho estudio presenta evidencia que demuestra que una persona medianamente versada en la material técnica no esperaría que los compuestos reivindicados (sustitución desde la posición 3) tuvieran las propiedades de sus isómeros de las posiciones 5 y 6.

Cuando se comparan las fórmulas estructurales para el isómero de posición 3 con las de los isómeros de posición 5 y 6, se reconoce que las interacciones entre los heteroátomos de la estructura bicíclica de indazol y los heteroátomos de la estructura azabicyclo serán claramente diferentes para cada isómero.

Detallando la topología molecular, se puede ver que en el isómero de la amida de posición 3 los heteroátomos están dispuestos más próximos en comparación con la disposición de los heteroátomos para los isómeros de la amida de posición 5 y 6. Como resultado, el potencial electrostático para el isómero de posición 3 es claramente diferente con respecto al de los isómeros de posición 5 y 6. Resultados similares se esperaría para los compuestos de tioamida correspondientes (cuando X es S).

Un aspecto particular de la topología molecular de los isómeros de amida consiste en el ángulo diedro formado por el enlace del grupo amida a la estructura del indazol. (diapositiva 8 del anexo 2) Para los isómeros de posición 3, dicho ángulo al ser determinado (por software de modelado) resulta ser bastante pequeño, mientras que para las posiciones 5 y 6 es relativamente grande, lo cual también aplica para las tioamidas. Entonces, con base en éstas observaciones, la persona versada en la materia técnica no esperaría que el isómero de posición 3 tuviera propiedades similares a las que presentan los isómeros de amida de posiciones 5 y 6.

En cuanto al método de síntesis de la presente solicitud, el mismo no es sugerido por D1 ni D3, donde se parte de compuestos que poseen el grupo acilo en la posición final 5 o 6 en que se tendrá la unión al azabicyclo tal como el fumarato de N-[(3R)-1-azabicyclo[2.2.2]oct-3-il]-1H-indazol-carboxamida, el cual se obtiene de la reacción del ácido 3-amino-4-metilbenzoico para reaccionar con ter-butil mercaptano en presencia de nitrito de sodio, y así producir un compuesto con el anillo de 5 miembros ácido 3-[(E)-(ter-butiltio)diazetil]-4-metilbenzoico, el cual a su vez se cicliza al reaccionar con terbutóxido de

potasio. (Diapositiva 11, anexo 2) Mientras tanto, en la presente solicitud se obtiene un compuesto de ácido 3-indazolcarboxílico como se puede observar en la diapositiva 10 del anexo 2.

Dados los argumentos anteriores, resulta claro que los compuestos reivindicados no se derivan del arte previo, y que ni D1 ni D3 sugerirían a la persona versada en la materia cómo modificar los compuestos de D1 de tal forma que se obtuvieran los de ésta invención, ya que se tiene rutas de síntesis distintas, y los compuestos difieren en su estructura. Además, presentan una significativa actividad biológica constituyendo una alternativa en el tratamiento de enfermedades asociadas con mal funcionamiento de los receptores nicotínicos.

Con el fin de mostrar que no es posible afirmar que los compuestos de la presente invención por ser similares a los del arte previo tengan la misma actividad biológica, presento copia del artículo ***“Similarity and Dissimilarity: A Medicinal Chemist’s View, Hugo Kubinyi, Perspectives in Drug Discovery and Design, 9/10/11: 225–252, 1998, Kluwer Academic Publishers”***, donde se describen modificaciones estructurales menores de compuestos análogos que pueden destruir la actividad biológica esperada. Adicionalmente, en este artículo Hugo Kubinyi describe compuestos inhibidores de termolisina que son similares, **pero con los cuales, a pesar de tener pequeñas modificaciones estructurales, se obtiene en un efecto biológico diferente, y también se muestran efectos opuestos a los esperados que pueden tener enantiómeros de barbituratos.**

En el artículo de Hugo Kubinyi se resalta un fenómeno bien documentado en el estado del arte, y es que **la mera similitud estructural entre compuestos no necesariamente indica que tengan una misma actividad biológica.** A partir de lo anterior es posible concluir que la presente invención no es obvia a la luz del arte previo establecido por el examinador, por cuanto que una persona del arte no sería conducida por estos para llegar a la misma.

Adicionalmente, las modificaciones estructurales no pueden ser consideradas obvias, ya que no existe información disponible sobre el impacto que tales cambios pueden tener sobre la actividad farmacológica enlace al receptor, actividad farmacocinética, o selectividad para un receptor específico. Por tanto, sin ninguna enseñanza o sugerencia del impacto de las sustituciones específicas sobre la estructura base para los compuestos reivindicados, debe reconocerse el nivel inventivo de la presente invención sobre los documentos D1 y D2.

Habiendo quedado sustentado el nivel inventivo de la solicitud, solicito a su Despacho que revoque la resolución 11751 y se otorgue el privilegio de patente a la invención titulada **“INSAZOLES, BENZOTIAZOLES, BENZOISOTIAZOLES, BENZISOXAZOLES Y PREPARACION Y USOS DE ESTOS”**.

#### 4. FUNDAMENTOS DE DERECHO

Fundamento el presente recurso en las siguientes normas:

- Artículo 18 de la Decisión 486 de la Comisión de la Comunidad Andina

#### 5. PETICIÓN

Solicito a su Despacho que con fundamento en los hechos y normas citadas:

1. Se revoque la resolución No. **11751** del 29 de febrero de 2012 en cuanto a la negación del privilegio de patente de invención.
2. Que como consecuencia de la revocación, se otorgue el privilegio de patente a la invención titulada **“INDAZOLES, BENZOTIAZOLES, BENZOISOTIAZOLES, BENZISOXAZOLES Y PREPARACION Y USOS DE ESTOS”**.

#### 6. PRUEBAS

6.1. Solicito que se tengan como tales, los documentos contenidos en el Expediente No. **06.108.716** de la referencia, en el que se presentó la solicitud de privilegio de patente para la invención titulada **“INDAZOLES, BENZOTIAZOLES, BENZOISOTIAZOLES, BENZISOXAZOLES Y PREPARACION Y USOS DE ESTOS”** a nombre de la sociedad **MEMORY PHARMACEUTICALS CORPORATION**.

6.2. **Anexo 1:** Datos que demuestran la actividad de los compuestos de la presente solicitud.

6.3. **Anexo 2:** diapositivas con el estudio comparativo entre isómeros de posición 3, 5 y 6.

6.4. **Anexo 3:** Similarity and Dissimilarity: A Medicinal Chemist's View, Hugo Kubinyi, Perspectives in Drug Discovery and Design, 9/10/11: 225–252, 1998, Kluwer Academic Publishers

**7. NOMBRE Y DIRECCION DEL RECURRENTE**

MEMORY PHARMACEUTICALS CORPORATION.  
100 Philips Parkway, Montvale,  
NJ 07645, ESTADOS UNIDOS DE AMERICA

**8. NOTIFICACIONES**

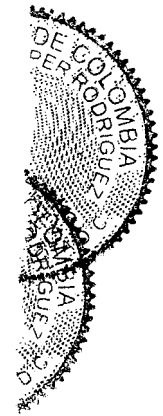
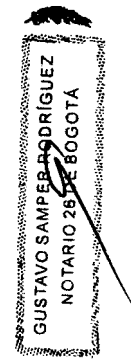
Las recibiré en la Secretaría de su Despacho y en mí oficina en la **Calle 94 A No. 7 A-09**,  
teléfono 7560770.

Del Señor Superintendente, Atentamente,

  
Margarita Castellanos Abondano

T.P.A. No. **70819** del C. Sup. de la Judicatura.  
C.C. No. **39.779.654** de Usaquén  
Calle 94 A No. 7 A-09  
Teléfono: 7560770 – FAX 7550158

Bogotá, D.C. **10 ABR. 2012**



**PRESENTACIÓN PERSONAL DE ABOGADO**

El anterior memorial fue presentado personalmente por

Margarita Castellanos Abandano —  
quien se identificó con la C. de C. No. 39779654 —

de Bogotá —

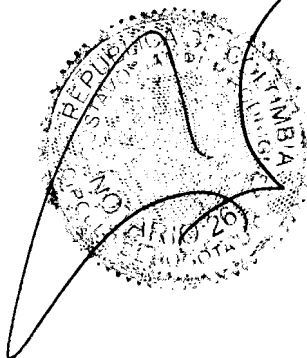
Tarjeta Profesional No. 70896 C.S.J. —

Bogotá, D.C. 10 ABR. 2012

El Notario Veintiséis

**Gustavo Samper Rodríguez**

Margarita Castellanos



**Datos de actividad**

Los siguientes datos demuestran la actividad de los compuestos reclamados. La tabla muestra las actividades de unión de los compuestos de la reivindicación 2 con base en los ensayos descritos en el ejemplo 95 de la presente solicitud.

| Actividad de unión (EC <sub>50</sub> , nm) | Nombre del compuesto                                                                                                           |
|--------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------|
| 1.46                                       | N-[(3 <i>R</i> )-1-Azabicyclo[2.2.2]oct-3-yl]-5-ethynyl-1H-indazole-3-carboxamide hydroformate                                 |
| 0.96                                       | N-[(3 <i>R</i> )-1-Azabicyclo[2.2.2]oct-3-yl]-5-phenoxy-1H-indazole-3-carboxamide                                              |
| 25.9                                       | N-[(3 <i>R</i> )-1-Azabicyclo[2.2.2]oct-3-yl]-6-(pent-1-yn-1-yl)-1H-indazole-3-carboxamide                                     |
| 21.84                                      | N-[(3 <i>R</i> )-1-Azabicyclo[2.2.2]oct-3-yl]-6-(phenylethynyl)-1H-indazole-3-carboxamide                                      |
| 0.04                                       | N-[(3 <i>R</i> )-1-Azabicyclo[2.2.2]oct-3-yl]-6-ethynyl-1H-indazole-3-carboxamide                                              |
| 0.37                                       | N-[(3 <i>S</i> )-1-Azabicyclo[2.2.2]oct-3-yl]-5-ethynyl-1H-indazole-3-carboxamide hydroformate                                 |
| 0.55                                       | N-[(3 <i>S</i> )-1-Azabicyclo[2.2.2]oct-3-yl]-6-(prop-1-yn-1-yl)-1H-indazole-3-carboxamide                                     |
| 60.1                                       | N-[(3 <i>S</i> )-1-Azabicyclo[2.2.2]oct-3-yl]-6-[2,2,2-trifluoro-1-hydroxy-1-(trifluoromethyl)ethyl]-1H-indazole-3-carboxamide |
| 0.07                                       | N-[(3 <i>S</i> )-1-Azabicyclo[2.2.2]oct-3-yl]-6-ethynyl-1H-indazole-3-carboxamide                                              |

Anexo 2: Diapositivas con el estudio  
comparativo entre isómeros de  
posición 3, 5 y 6.

13



**US Application No. 10/669,645**  
**12/11/06 Office Action**

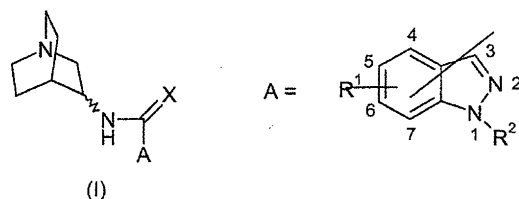
**March 12, 2007**

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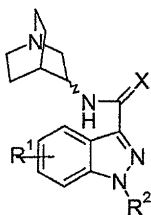
# Memory proposed claims

## Present claim 1:



*Azabicyclo is attached to A, an indazole, at any of positions 3, 4, or 7*

## Amended claim 1:

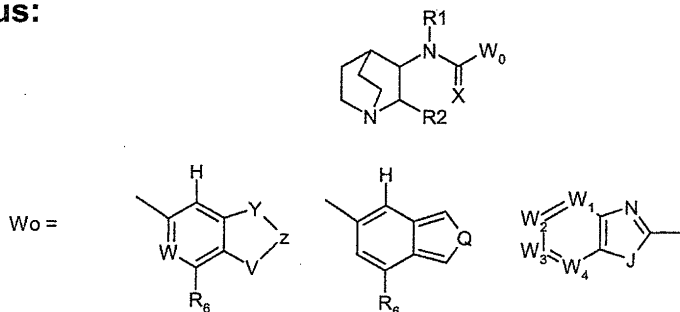


*Azabicyclo is attached at the 3 position of A, an indazole*

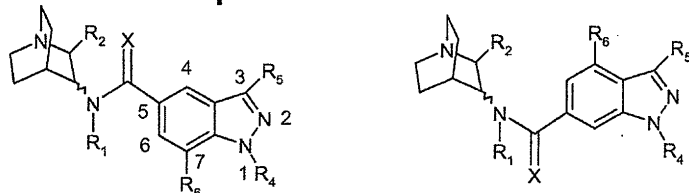
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**Broad genus:**



**Subgenus of indazole compounds:**



*Azabicyclo group attached to the 5 or 6 position of the indazole (not 3 position)*

*[V-Z-Y is N(R4)-N=C(R5) or C(R5)=N-N(R4)]*

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## Rejection

- Rejection is based on structural obviousness
- Structural obviousness is based on the presumption that structurally similar isomers (positional isomers) will have similar properties
  - ▶ In re Norris cited in Office Action
- Presumption is not supported by the evidence in this case
  - ▶ Substitution at 3-position vs. 5- or 6-position of an indazole ring

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## Evidence does not support presumption

- Evidence in this case does not support the presumption that the positional isomers involved have similar properties
- Evidence available for analysis
  - ▶ Prior art disclosure
  - ▶ Chemical characteristics
  - ▶ Molecular topology
  - ▶ Synthetic methods and intermediates

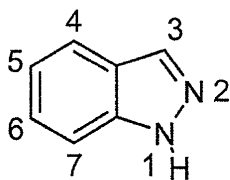
***Based on the evidence, one of ordinary skill in the art would not expect that the 3-positional isomers in this case would have similar properties compared to 5- or 6-positional isomers***

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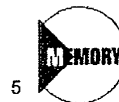


## 3-position vs. 5- or 6-position: Prior art disclosure

- The Walker et al. genus includes azabicyclo groups attached to a fused bicyclic ring structure containing a 6-membered ring and a 5-membered ring
  - ▶ Subgenera include the possibility of the azabicyclo group being attached to a 5-membered ring, however all indazoles limit attachment to 6-member ring at the 5- and 6-positions
- The Walker et al. genus does not encompass or suggest the Memory 3-position indazole isomers, in which the azabicyclo group is attached to the 5-membered ring

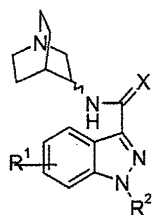


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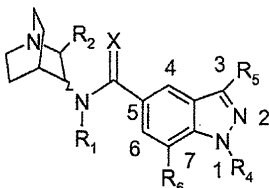


## 3-position vs. 5- or 6-position: Chemical characteristics

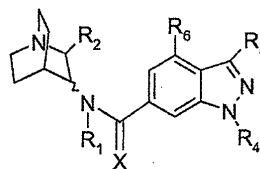
- The Memory 3-position compounds include an azabicyclo group attached to a heteroaryl ring (5-membered ring)
- In contrast, Walker et al.'s 5- or 6-position compounds include an azabicyclo group attached to a phenyl ring (6-membered ring)
- The interaction between the heteroatoms of the azabicyclo group and the hetero atoms of the fused bicyclic ring will clearly be different for a 3-position compound as compared to 5- or 6-position compounds



Memory  
3-position



Walker et al.  
5-position



Walker et al.  
6-position

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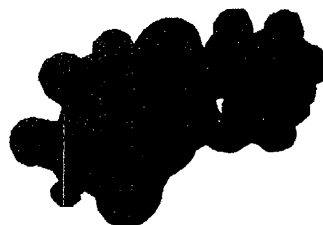
## 3-position vs. 5- or 6-position: Molecular topology



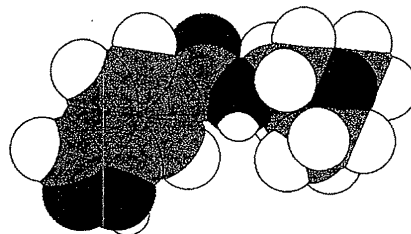
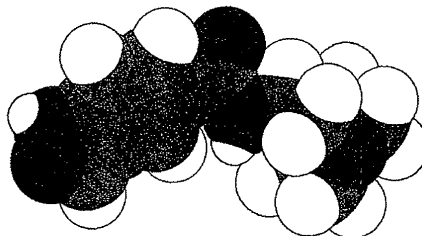
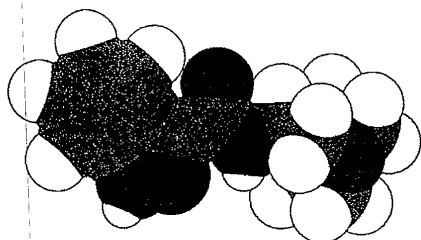
**C(3)-Amide  
Electrostatic Potential**



**C(5)-Amide  
Electrostatic Potential**



**C(6)-Amide  
Electrostatic Potential**



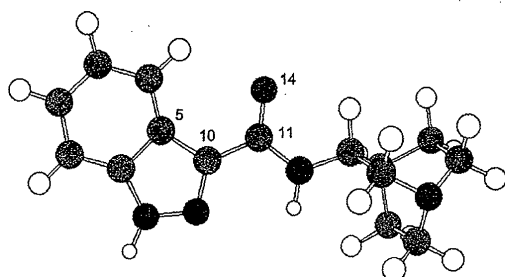
Semi-empirical energy minimized conformation calculations performed at AM1 Theory level with closed shell and Mulliken charges to a minimum RMS gradient of 0.100 using Chem 3D (CambridgeSoft). Analysis carried out with fixed R-enantiomers.

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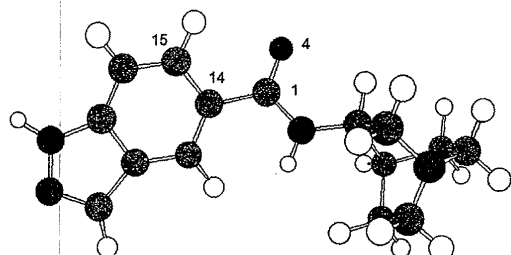


# 3-position vs. 5- or 6-position: Molecular topology



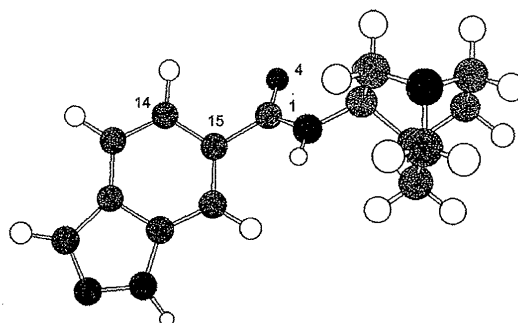
**C(3)-Amide**

C(5)-C(10)-C(11)-O(14)  
Dihedral Angle -0.448 °



**C(5)-Amide**

C(15)-C(14)-C(1)-O(4)  
Dihedral Angle -36.261 °



**C(6)-Amide**

C(14)-C(15)-C(1)-O(4)  
Dihedral Angle -41.748 °

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## 3-position vs. 5- or 6-position: Molecular topology summary

| Topology characteristics  | Memory                          | Walker et al.               |                             |
|---------------------------|---------------------------------|-----------------------------|-----------------------------|
|                           |                                 | 5-position                  | 6-position                  |
| Position on indazole ring | 3-position                      | 5-position                  | 6-position                  |
| Azabicyclo attached to    | heteroaryl ring<br>(5-membered) | phenyl ring<br>(6-membered) | phenyl ring<br>(6-membered) |
| Electrostatic potential   | Close proximity                 | Distant<br>proximity        | Distant<br>proximity        |
| Carbonyl dihedral angle   | -0.448 °                        | -36.261 °                   | -41.748 °                   |

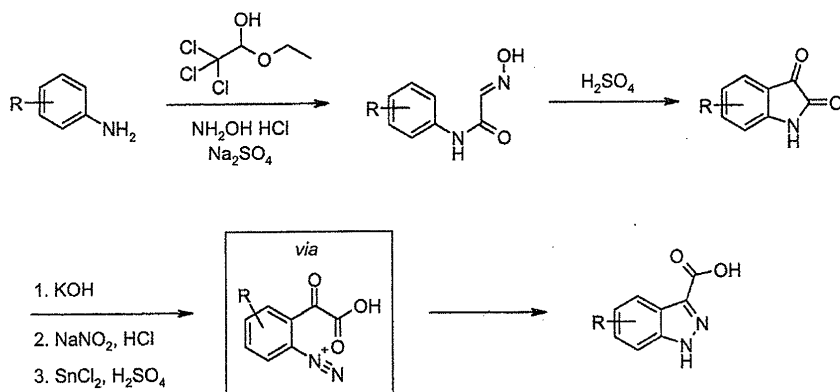
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## 3-position vs. 5- or 6-position: Synthetic methodology

### Synthesis of 3-position carboxylic acid intermediate



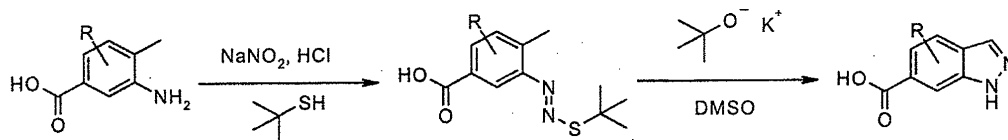
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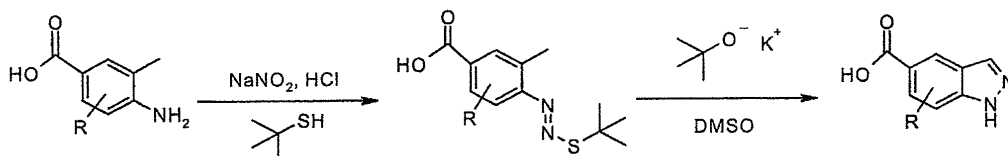


## 3-position vs. 5- or 6-position: Synthetic methodology

### Synthesis of 6-position carboxylic acid intermediate (Walker et al.)



### Synthesis of 5-position carboxylic acid intermediate



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## Conclusion

- 3-position indazole isomers are not obvious over 5- and 6-position indazole isomers
- Evidence does not support the presumption that the positional isomers involved in this case have similar properties
  - ▶ Prior art disclosure
  - ▶ Chemical characteristics
  - ▶ Molecular topology
  - ▶ Synthetic methods and intermediates
- One of ordinary skill in the art would not expect that the 3-positional isomers in this case would have similar properties compared to 5- or 6-positional isomers

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**Anexo 3: copia del artículo**

**“Similarity and Dissimilarity: A Medicinal Chemist’s View, Hugo Kubinyi, Perspectives in Drug Discovery and Design, 9/10/11: 225–252, 1998, Kluwer Academic Publishers”**



## Similarity and Dissimilarity: A Medicinal Chemist's View

**Hugo Kubinyi**

*Drug Design, BASF AG, D-67056 Ludwigshafen, Germany*

Several 3D approaches discussed in this volume describe methods for the analysis and quantitative description of chemical similarity. The underlying concept is that chemical similarity is reflected by similar biological activities — i.e. chemically closely related analogs should be related in their mode of action, as well as in their relative potencies. This fundamental assumption has, indeed, been used in medicinal chemistry research, and has led to many valuable drugs.

However, chemical similarity may have different facets if a computer chemist or a medicinal chemist look at the compounds. There is no argument that for maximal affinity a ligand of a biological macromolecule has to fit the binding pocket geometrically and that hydrophobic surfaces of the ligand and the binding site have to be complementary. The functional groups of the ligand need a separate consideration. For lipophilicity, there is no significant difference between, e.g. –O– and –NH– in an organic molecule; for ionization, there is a big difference whether the nitrogen atom is part of a basic group (an amine) or a neutral group (e.g. in an amide); and for binding, potency differences of several orders of magnitude may result from the exchange of the hydrogen bond acceptor –O– against a donor function –NH–.

### 1. Similarity as a Design Principle in Lead Optimization

Nearly all drugs result from the optimization of a lead structure. Sources of such leads are natural products from plants or microorganisms, synthetic chemicals or their intermediates, hits from (high-throughput) screening of in-house and combinatorial libraries, rational concepts from a biochemical pathway or the unexpected observation of a therapeutically useful side-effect of a drug. Most often, the biological activity of a lead structure is neither optimal, with respect to its efficacy, nor with respect to specificity, bioavailability, pharmacokinetics, toxic and other side-effects. Chemists perform more or less systematic variations of lead structures, using the experience of about 100 years of medicinal chemistry and the results of (quantitative) structure–activity relationships.

The principle of bioisosteric replacement of functional groups serves as a successful optimization strategy [1–3]. Its systematic application has resulted in a broad variety of therapeutically used drugs, many of them finally having the desired combination of favorable properties. A few examples of typical but different consequences of isosteric replacement of atoms or groups are illustrated by compounds 1–3 (Fig. 1), some others with unexpected effects on biological activities are discussed in later sections of this chapter.

In their attempts to optimize lead structures, medicinal chemists intuitively follow the principles of evolution. In genetic and evolutionary algorithms, randomly generated starting models (the lead structures) are reproduced involving random mutations and crossover (the chemical variation of the structures). Better models (compounds) are kept for further modification; worse ones are discarded. The biological activity, in later

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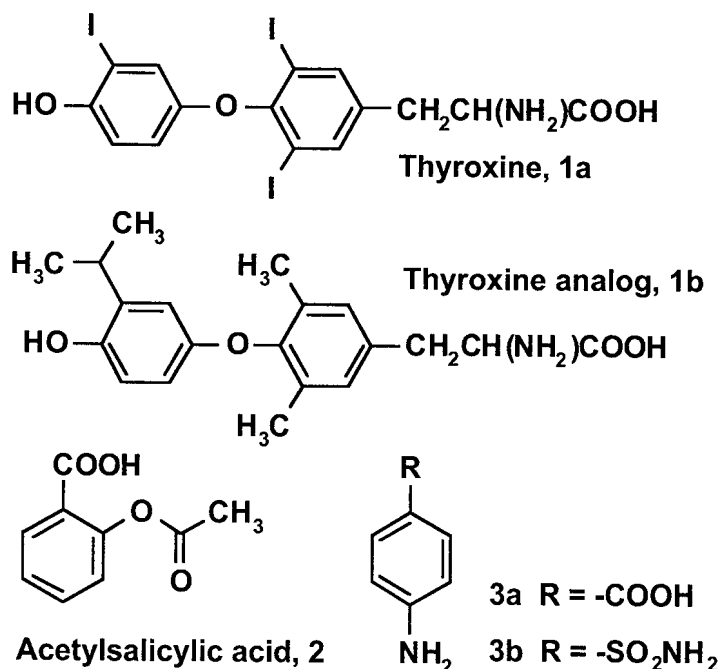


Fig. 1. Different effects of isosteric replacement. (a) The substitution of all three iodine atoms of thyroxine **1a** leads to an analog **1b** which still acts as a thyromimetic agent. (b) Replacement of an ester function,  $-O-CO-$ , by an amide function,  $-NH-CO-$ , most often produces analogs with higher metabolic stability; if this replacement is done in acetylsalicylic acid **2**, an inactive analog results because the amide is no longer able to transfer an acetyl group to a certain serine residue of cyclooxygenase. (c) *p*-Aminobenzoic acid **3a** is an essential metabolite of microorganisms; if the acid group,  $-COOH$ , is replaced by a sulfonamide group,  $-SO_2NH_2$ , the antimetabolite sulfanilamide **3b** results (active metabolite of the antibacterial sulfonamide sulfamidochrysoidine).

stages a selectivity index or some other biological property, serves as the 'fitness function' for the 'survival' of certain structural entities. That genetic and evolutionary algorithms, indeed, reduce the effort in searching the most active analogs has recently been confirmed by dedicated investigations (e.g. [4,5]).

## 2. The Biological Activity of a Ligand Depends on its Flexibility

Other common structural modifications in the optimization of a lead structure are the dissection of rings or the rigidification of flexible molecules. Molecules with several rotatable bonds may adopt many different geometries — some of them being favorable because of low internal energies, others being less favorable because of van der Waals or electrostatic repulsions between non-bonded atoms or groups. If different conformations of such molecules are 'frozen' by closing rings between certain atoms, either one of two very different consequences results. If the frozen conformation differs from the

bioactive conformation of the flexible lead or if the added atoms interfere with the binding, biological activity will be more or less destroyed. If the ring closure stabilizes the bioactive conformation, usually a significant increase in biological activity results. This comes from the fact that the binding of a flexible analog is entropically unfavorable, due to a loss of rotational degrees of freedom, whereas the rigid analog has already lost its flexibility prior to binding. Two examples of highly similar, flexible and rigid analogs are the pairs 4 and 5 [6] and 6 and 7 [7], respectively, where the rigid analogs are significantly more active than their flexible counterparts (Fig. 2).

The computer program CAVEAT was developed for the design of rigid analogs which bear a pharmacophore in a certain geometry [8]. CAVEAT starts from a struc-

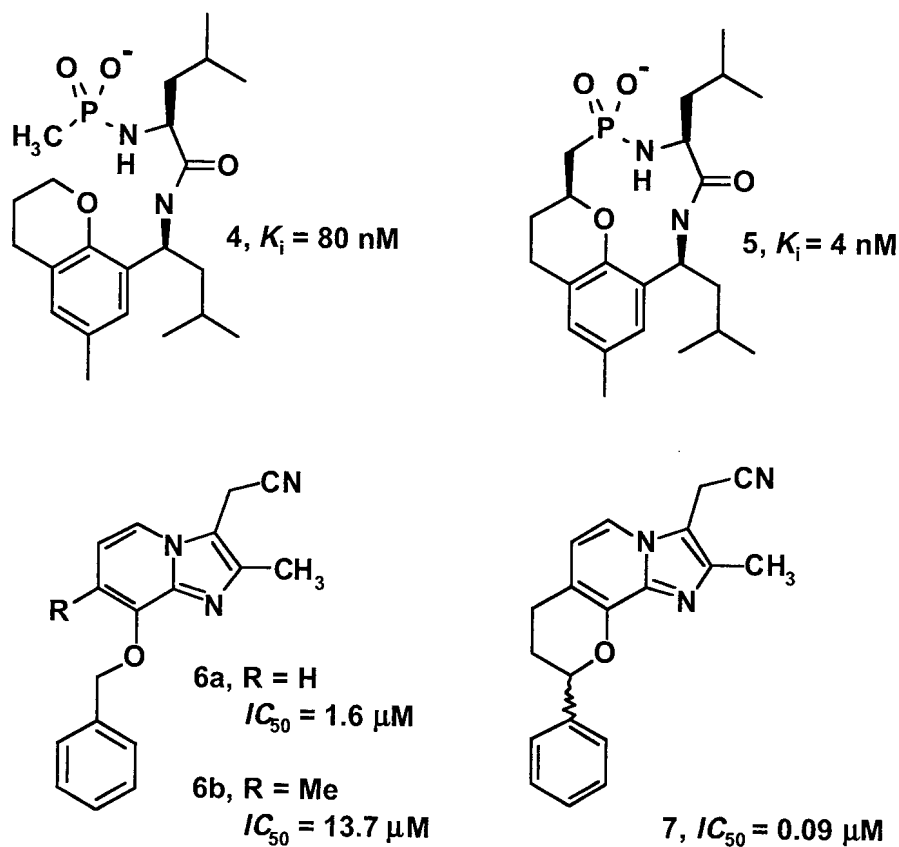


Fig. 2. Rigidification of a bioactive conformation significantly increases biological activities. (a) If an additional ring is introduced into the thermolysin inhibitor 4 to produce analog 5, a 20-fold increase in affinity is observed. (b) Introduction of a methyl group into the  $\text{H}^+/\text{K}^+$ -ATPase inhibitor 6a reduces biological activity by a factor of 8, most probably due to a destabilization of the bioactive conformation; if the substituent R in 6b is extended to a new ring (compound 7), which fixes the bioactive conformation, the biological activity of 6b is enhanced by a factor of 150.

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tural hypothesis or from the known 3D structure of a ligand, e.g. a polypeptide, and extracts vectors of residues that participate in binding. In a peptide, these vectors are e.g. the  $C_\alpha$ – $C_\beta$  bonds of the amino acid side chains. Then the program identifies ring systems that are suited to accommodate these residues in exactly the same relative geometries.

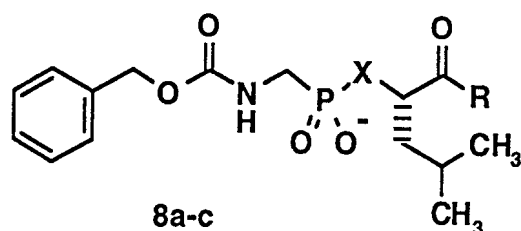
A lead structure, where a significant increase in binding affinity is achieved by steric constraints, not by ring closure, has been described by Kaplan and Bartlett [9]. In the flexible phosphonate tripeptide Cbz–Phe–Ala[PO<sub>2</sub><sup>–</sup>]<sup>–</sup>–O–CH(CH<sub>3</sub>)COOH (= Z–Phe–Ala<sup>P</sup>–(O)–Ala; <sup>P</sup> indicates a PO<sub>2</sub><sup>–</sup> residue instead of a C=O group), inhibitory activities against the zinc protease carboxypeptidase A increase significantly with the stepwise introduction of bulky residues. In the series Z–Phe–Ala<sup>P</sup>–(O)–Ala, Z–Phe–Ala<sup>P</sup>–(O)–Phe and Z–Phe–Val<sup>P</sup>–(O)–Phe, affinities increase from 56 nM to 0.001 nM and 0.000010–0.000027 nMol (i.e. 10–27 fMol). The more active analogs have a higher lipophilicity, due to the additional phenyl group in phenylalanine, as compared to alanine, and due to the isopropyl group instead of the methyl group of the C-terminal lactic acid residue. But in this case, the affinity difference of about six orders of magnitude cannot be attributed only to the increase in lipophilicity which changes by less than three orders of magnitude. It must also result from the conformational constraint imposed on both phenylalanines by the central valine and which, by fortune, stabilizes the bioactive conformation. In terms of ‘chemical similarity’, all three analogs are closely related; with respect to their internal flexibility and their preferred conformations, they are not.

### 3. Biological Potencies of Similar Molecules

For compounds with comparable flexibility, most often similar analogs have also similar biological potencies. That this need not always be the case can be seen by a comparison of three thermolysin inhibitors **8a–c** (Fig. 3) [10,11]. All analogs **8a** and **c**, with X = –NH– and –CH<sub>2</sub>–, are about 1000 times more potent than the X = –O– analogs **8b** (R = OH or different amino acids). The explanation for this effect can be easily derived from the 3D structure of the complex of thermolysin with the inhibitor **8a** (R = –Leu–OH). If X is an –NH– group (series **8a**), a hydrogen bond is formed between this group and the oxygen atom of an alanine carbonyl group. In the –O– analog **8b**, this hydrogen bond cannot be formed; in addition, an electrostatic repulsion between the two oxygen atoms results. The biological activity of the –CH<sub>2</sub>– analog **8c** has been predicted [12] to be comparable to the –NH– analogs and much higher than for the –O– analogs. This was later confirmed by the synthesis of the inhibitors **8c** [11].

A similar but less pronounced effect is observed for the thrombin inhibitors **9a–c** (Fig. 4) [13]; in this case, the non-bonded contact between the –X– group of the ligand and the carbonyl group of Gly 216 in the binding site of thrombin is responsible for the structure–activity relationship. If, on the other hand, the carbonyl group of **10a**, which forms a hydrogen bond with the –NH– group of Gly 216, is replaced by –CH<sub>2</sub>– (**10b**), the affinity is reduced by more than three orders of magnitude (Fig. 4) [13].

Neutral endopeptidase 24.11 (NEP 24.11; enkephalinase) is a zinc protease with some homology to thermolysin. The NEP inhibitors **11a–d** (Fig. 5) show a different

Binding constants  $K_i$  in  $\mu\text{M}$ 

| R       | 8a<br>X = -NH- | 8b<br>-O- | 8c<br>-CH <sub>2</sub> - |
|---------|----------------|-----------|--------------------------|
| -OH     | 0.76           | 660       | 1.4                      |
| -Gly-OH | 0.27           | 230       | 0.3                      |
| -Leu-OH | 0.01           | 9         | 0.01                     |

Fig. 3. The X = -NH- group of the thermolysin inhibitors **8a** forms a hydrogen-bond with the carbonyl oxygen atom of Ala 113; this affinity-enhancing effect is only moderated by desolvation of the ligand in going from the free to the bound state. All oxygen analogs **8b** are much less active, because they cannot form such a hydrogen-bond; in addition, there is the unfavorable desolvation effect and an electrostatic repulsion between the oxygen atoms of the ligand and the binding site. Like the -O- analogs **8b**, the -CH<sub>2</sub>- analogs **8c** cannot form the hydrogen-bond to Ala 113, but this disadvantage is counterbalanced by the effect that no desolvation of the CH<sub>2</sub> group of the ligand and no electrostatic repulsion take place. The differences between X = -NH-, -O- and -CH<sub>2</sub>- are identically observed whether R = -OH, -Gly-OH or -Leu-OH.

structure-activity relationship, as compared to the preceeding examples. Here the lipophilic -CH<sub>2</sub>- and -S- analogs are much less active than the -NH- and -O- analogs, which both have comparable biological activities [14]. As long as the 3D structure of NEP 24.11 remains unknown, no explanation can be given for this effect.

An unexpected structure-activity relationship has been observed for the macrocyclic renin inhibitors **12a** and **b** (Fig. 5) [15]. Modelling of the complex of **12a** with the aspartyl protease renin indicated that X = -NH- should form a hydrogen bond with one of the Asp 226 oxygen atoms. Thus, the replacement of -NH- by -O- should reduce the affinity. The opposite was observed; the affinity of analog **12b** is increased by more than two orders of magnitude. The authors discuss several possible explanations for this observation but finally conclude: 'we have attempted to find a plausible basis for this surprising reversal in potency, but without much success ... the comparison [of both analogs] is therefore intrinsically more difficult, and will constitute a more demanding test for thermodynamic simulation techniques [as compared to the thermolysin inhibitors, Fig. 3]'.

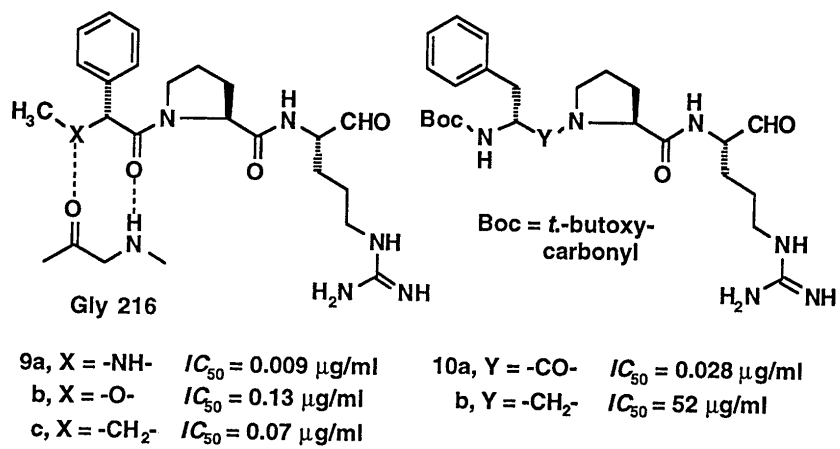


Fig. 4. The inhibitor **9a** forms two hydrogen-bonds with glycine 216 of the serine protease thrombin. As compared to the thermolysin inhibitors **8a-c** (Fig. 3), a similar but much less pronounced effect is observed by structural variation of the group *X* in the thrombin inhibitors **9a-c**. If, on the other hand, the carbonyl group of **10a** is replaced by a methylene group, the resulting pseudopeptide **10b** shows a 2000-fold weaker inhibitory activity.

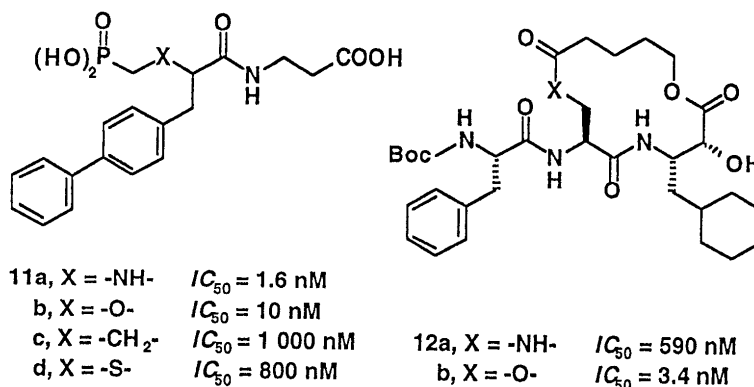


Fig. 5. The NEP 24.11 inhibitors 11a and b are much more potent than the more lipophilic analogs 11c and d. The macrocyclic renin inhibitor 12a is less potent than its oxygen analog 12b, although its NH group should form a favorable hydrogen-bond with the carboxylate of Asp 226 of the enzyme.

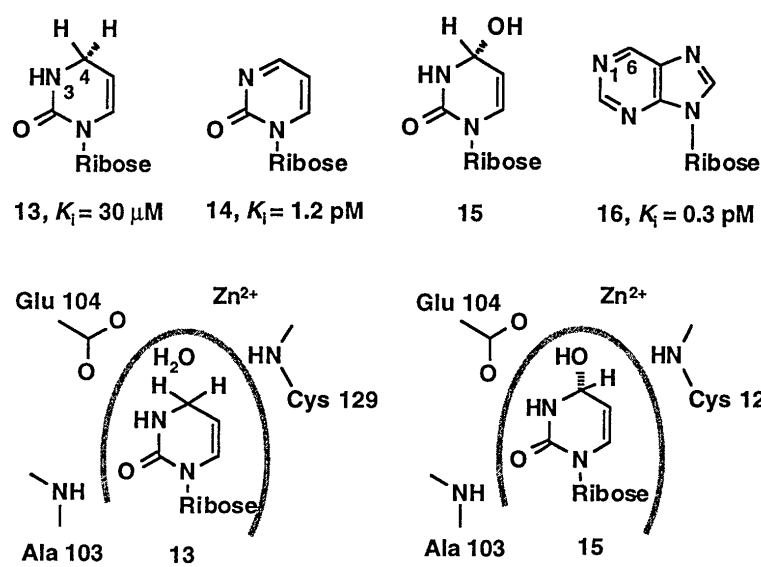


Fig. 6. 3,4-Dihydrozebularine 13 is a weak inhibitor of cytidine deaminase. Zebularine 14 interacts with the enzyme as the 3,4-hydrate 15, as confirmed by X-ray structure analysis. The favorable interactions of the hydroxy group enhance the biological activity by more than seven orders of magnitude. Nebularine 16, a strong adenosine deaminase inhibitor, is also proposed to interact as the 1,6-hydrate.

There are many examples in literature where the introduction of an –OH group into a ligand either causes an increase or a decrease of biological affinities. From a theoretical point of view, this is not surprising. If the new –OH group forms hydrogen bonds with polar groups at the binding site (either as a hydrogen bond donor or as an acceptor), the net free energy depends on the balance between the desolvation energies of the water shells at the surfaces of the ligand and the binding site, as compared to the energy of the formed hydrogen bond/s and the entropy gain by the release of some water molecules. Certain tightly bound water molecules in the binding cavity of a protein (usually seen in the X-ray structures), e.g. those which form more than two hydrogen bonds to the protein, are not easily removed. The attempt to introduce an –OH group into the ligand, to replace such a water molecule, must necessarily fail.

An example where this is not the case, and where significantly enhanced binding affinities result after the introduction of such a hydroxy group, are the cytidine and adenosine deaminase inhibitors **13–16** (Fig. 6) [16,17]. In this special case, the resulting affinity differences are 7 to 8 orders of magnitude!

#### 4. Biological Potencies *versus* Similar Biological Targets

Functionally corresponding proteins from different species have identical or very similar 3D structures, but they normally differ in their amino acid composition. Although they always show, dependent on the evolutionary relationship between the two species, a certain degree of homology, structure–activity relationships may significantly differ, even after the replacement of just one single amino acid by another one. This has been shown, for example, by a comparison of rat and human 5-HT receptors [18]. Although both have about 90% identity and more than 95% homology in their amino acid sequences, the binding affinities of a series of 5-HT receptor ligands and  $\beta$ -adrenergic blocking agents are not related ( $n = 10$ ;  $r = 0.27$ ). If one (!) amino acid of the human receptor, i.e. threonine 355, is replaced by the corresponding amino acid of the rat receptor, i.e. asparagine, the binding affinities of the ligands change significantly. Now the human mutant 5-HT receptor behaves like a rat receptor; all affinities to both receptors are more or less identical ( $r = 0.98$ ) [18].

In the past, new compounds have always been tested in animals before they could be applied to humans. Human proteins were not available, except in rare cases. Only for those proteins that could be extracted from human material, e.g. hemoglobin or thrombin from blood, could one predict the human biological activity of a new drug from *in vitro* studies, prior to animal studies. With the progress in gene technology, it is now possible to produce human proteins in sufficient quantities to establish test models. Thus, their biological activity in humans can be forecasted from investigations at the molecular level. How important this is, can be illustrated, e.g. by the activities of the renin inhibitor remikiren **17** against the renins of different species (Fig. 7) [19].

Different QSAR models for dihydrofolate reductase (DHFR) inhibitors, chemically related to the antibacterial drug trimethoprim **18** (Fig. 8), describe the inhibitory activities *versus* *E. coli* and *L. casei* DHFR [20]. Whereas in the case of *E. coli* DHFR, the 3-, 4- and 5-substituents at the benzyl group contribute to biological activities, only the

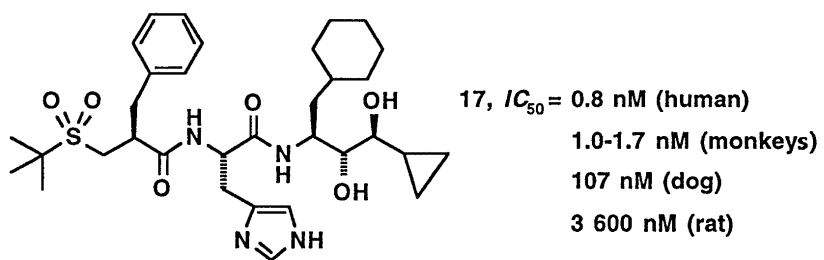
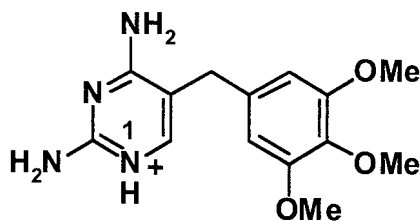


Fig. 7. Remikiren 17 is a much stronger inhibitor versus human and monkey renins, as compared to dog and rat renins. If this compound had only been tested in small laboratory animals (as was the case before gene technology could be applied in drug research), it would not have been considered for further development, due to its weak biological activity.



**Trimethoprim, 18**  
**(protonated form)**

Fig. 8. Trimethoprim **18** is proposed to interact with dihydrofolate reductase in its protonated form, where N1 changes its character from a hydrogen-bond acceptor to a hydrogen-bond donor. Due to the positive charge of the ligand, the exchange of negatively charged amino acids of the protein to neutral amino acids and of neutral to positively charged amino acids reduce the affinity of the ligand.

3- and 4-substituents are relevant for the activities against *L. casei* DHFR. Several years later, the X-ray analyses of both enzymes explained these differences: in *L. casei* DHFR, there is a much narrower pocket, due to a bulky leucine in the binding site, as compared to a flexible methionine side chain in *E. coli* DHFR [20].

Whereas many differences in the binding affinities to related biological targets can be attributed to different non-covalent interactions with the amino acids of the binding sites, such effects may also result from more distant changes in a protein. Trimethoprim **18** is thought to bind to DHFR in its positively charged form. An *E. coli* mutant, where the negatively charged glutamate residue 118 is replaced by a neutral glutamine, has a 4.5-fold lower affinity for trimethoprim, despite the fact that the modified group is about 15 Å apart from the charged N1 of trimethoprim. An even more pronounced effect is observed in a double mutant (Glu 118 Gln, Leu 28 Arg): the affinity of trimethoprim is reduced by a factor of 190, although the positive charge of the arginine residue is about 8 Å apart from the positive charge of the ligand [21]. This reduction of binding affinities has been used to explain the selectivity of 5 to 6 orders of magnitude of trimethoprim against bacterial DHFRs, as compared to avian and mammalian DHFRs. In chicken DHFR, seven amino acids in the environment of the binding site (not in the binding site itself) have changed charge from negative to neutral or from neutral to positive, as compared to *E. coli* DHFR. If one compares the effects observed in the *E. coli* double mutant with these figures, the changes seem to be sufficient to explain the observed effect [21].

Thiorphan **19** and its *retro-inverso* peptide, *retro*-thiorphan **20** (Fig. 9), are inhibitors of the structurally related zinc proteases thermolysin and NEP 24.11. Although the affinities of the ligand pair differ, from enzyme to enzyme, by three orders of magnitude, there are no significant differences between them [22]. Thus, they may be considered to be 'similar', which was also confirmed by the X-ray structure analyses of their complexes with thermolysin; both compounds bind in an analogous manner and have corresponding interactions with the protein [23]. On the other hand, their activities

NC(CS)C(=O)Cc1ccccc1  
Thiorphan, 19

NC(CS)C(=O)Cc1ccccc1  
*retro*-Thiorphan, 20

|             |                            |                            |
|-------------|----------------------------|----------------------------|
| Thermolysin | $K_i = 1.8 \mu\text{M}$    | $K_i = 2.3 \mu\text{M}$    |
| NEP 24.11   | $K_i = 0.0019 \mu\text{M}$ | $K_i = 0.0023 \mu\text{M}$ |
| ACE         | $K_i = 0.14 \mu\text{M}$   | $K_i > 10 \mu\text{M}$     |

NC(CS)C(=O)Cc1ccccc1  
21 a  
R =  $\alpha$ -H  
n = 1  
 $IC_{50}$  values

NC(CS)C(=O)Cc1ccccc1  
21b  
R =  $\alpha$ -H  
n = 0

NC(CS)C(=O)Cc1ccccc1  
21c  
R =  $\beta$ -H  
n = 0

|           |        |         |          |
|-----------|--------|---------|----------|
| NEP 24.11 | 1.1 nM | 11.5 nM | 2 820 nM |
| ACE       | 5.5 nM | 16 nM   | 11.5 nM  |

Fig. 9. Thiorphan 19 and retro-thiorphan 20 are 'similar' with respect to thermolysin and NEP 24.11, but they are highly 'dissimilar' with respect to angiotensin converting enzyme (ACE). Changing the configuration of 21a and b at the ring junction, from  $\alpha$ -H to  $\beta$ -H in 21c, does not influence the ACE-inhibitory activity, but reduces the NEP-inhibitory activity to a large extent; thus, the selectivity against ACE is increased by a factor of 300.

against yet another related zinc protease, angiotensin converting enzyme (ACE), are significantly different [22]; with respect to this enzyme, the analogs are 'dissimilar'. Whether the differences result from different binding modes or from an unfavorable binding geometry of *retro*-thiorphan in ACE will only be known if the ACE 3D structure becomes available. Corresponding differences in the structure–activity relationships are observed in some other dual zinc protease inhibitors **21a–c** (Fig. 9) [24]. Again, the degree of similarity and dissimilarity of the ligands depends on the biological target.

Corresponding problems are also observed in toxicity predictions for humans, as illustrated by the toxicities of lysergic acid diethylamide (LSD) and 2,3,7,8-tetrachlorodioxin ('dioxin') in different species. LSD is only weakly toxic to mice ( $LD_{50} = 50\text{--}60\text{ mg/kg}$ ) and rats ( $LD_{50} = 16.5\text{ mg/kg}$ ) but significantly more toxic to rabbits ( $LD_{50} = 0.3\text{ mg/kg}$ ). An elephant, which was (cautiously?) treated with an LSD dose of 0.3 g (corresponding to about 0.06 mg/kg), died within several minutes [25]! Humans seem to tolerate LSD quite well. Cases of death have been observed as the result of drug-induced suicides, but not by any toxicity of the drug itself.

2,3,7,8-Tetrachlorodioxin is highly toxic to several species — e.g. guinea-pigs ( $LD_{50} = 0.6\text{--}2.5\text{ }\mu\text{g/kg}$ ) and mink ( $LD_{50} = 4\text{ }\mu\text{g/kg}$ ). It is much less toxic for mice ( $LD_{50} = 114\text{--}280\text{ }\mu\text{g/kg}$ ), rats ( $LD_{50} = 22\text{--}320\text{ }\mu\text{g/kg}$ ), hamsters ( $LD_{50} = 1150\text{--}5000\text{ }\mu\text{g/kg}$ ), rabbits ( $LD_{50} = 115\text{--}275\text{ }\mu\text{g/kg}$ ), dogs ( $LD_{50} > 100$  and  $< 3000\text{ }\mu\text{g/kg}$ ) and monkeys ( $LD_{50} < 70\text{ }\mu\text{g/kg}$ ) [26]. If one extrapolates from the monkey, dioxin should be relatively 'harmless' for humans, at least if only acute toxicity is considered. However, the significantly different toxicity *versus* the closely related species guinea pig and hamster puts a caveat on too simple and straightforward extrapolations. Of course, for humans the  $LD_{50}$  is not the relevant endpoint, but rather the no-effect  $LD_0$  level!

## 5. Similar Structures and Activities, but Different Binding Modes

Comparable biological activities of chemically similar structures do not necessarily result from analogous binding modes of all analogs. Since at least in 3D QSAR, if not in all QSARs, the correct superposition of all analogs within a series is a precondition for reliable results, a better knowledge of the binding modes is most important. Unexpected differences in the binding modes of some analogs certainly produce problems in 3D QSAR studies that are based on such a mutual alignment of the structures. But it is to be expected that also 3D QSAR modifications, which do not require a structural alignment, should have problems in such cases.

Differences in binding modes have been extensively reviewed [1,27–29]. Thus, only some examples will be summarized here, without detailed discussion. Purine nucleoside phosphorylase (PNP) inhibitors **22** and **23** show surprising structure–activity relationships, which can be explained only by inspecting the X-ray structures of the inhibitor complexes (Fig. 10). The exchange of an acceptor nitrogen to a donor nitrogen atom changes not only the interactions with the directly involved asparagine side chain, but also of an 8-amino group with the threonine hydroxyl and methyl groups [30,31].

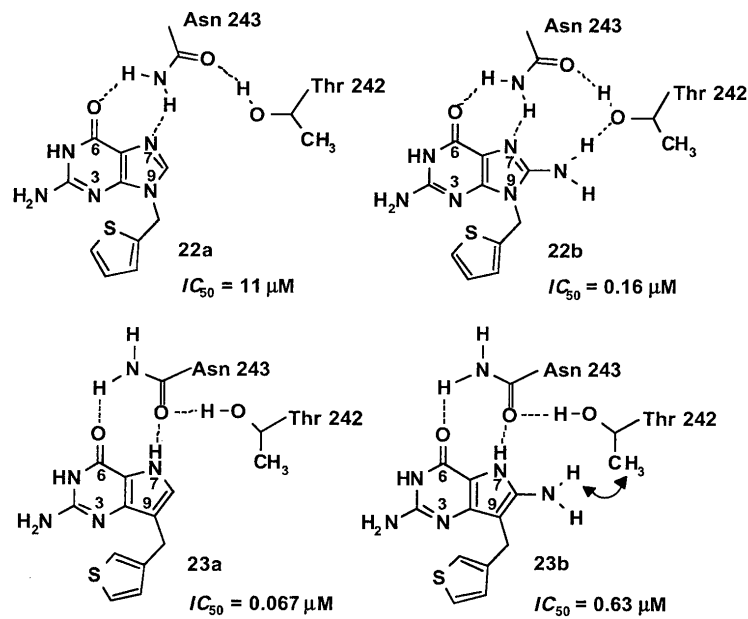


Fig. 10. The X-ray structures of the purine nucleoside phosphorylase (PNP) complexes with the inhibitors 22 and 23 show that 22a forms less favorable hydrogen-bonds with Asp 243 than the desazapurine 23a. On the other hand, the introduction of an 8-amino group has different consequences in 22a and 23a. Whereas the new amino group of 22b forms an additional hydrogen-bond to Thr 242, it causes unfavorable polar/nonpolar interactions in 23b.



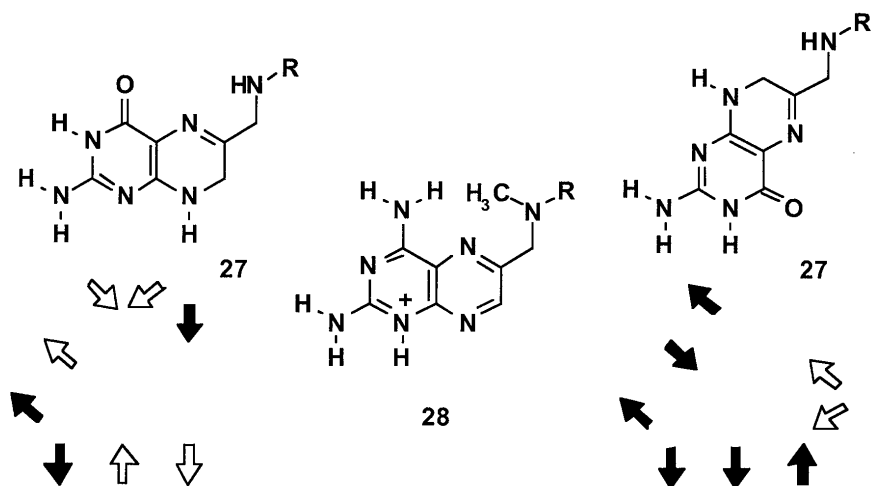


Fig. 12. Dihydrofolate 27 and the positively charged form of methotrexate 28 ( $R = p\text{-C}_6\text{H}_4\text{-CONHCH(COOH)CH}_2\text{CH}_2\text{COOH}$ ) have different patterns of hydrogen-bond donors and acceptors, if they are superimposed according to their ring systems (left side). If the ring system of 27 is rotated around the bond which connects the ring system with the rest of the molecule, a much better similarity of the hydrogen-bond patterns results (right side). Filled arrows indicate identical directions of the hydrogen-bonds in the analogs 27 and 28.

Carbobenzoxy-phenylalanine (Cbz-Phe) **24a** and  $\beta$ -phenylpropionyl-phenylalanine ( $\beta$ -PPP) **24b** (Fig. 11) differ only by their X group. Nevertheless, they bind to thermolysin in an opposite direction, as uncovered by the determination of the 3D structures of the complexes [32]. Reverse binding modes are also observed for some structurally related viral coat protein ligands — e.g. compounds **25** and **26** (Fig. 11) [33,34].

Dihydrofolate **27**, the substrate of dihydrofolate reductase (DHFR), and the DHFR inhibitor methotrexate **28** seemingly differ only in minor chemical details (Fig. 12). However, this small difference has significant consequences on the hydrogen-bond donor and acceptor patterns of the compounds. Accordingly, dihydrofolate and methotrexate accommodate the binding site of DHFR in different modes [35], an effect which has been predicted from the X-ray structure of the dihydrofolate/DHFR complex [36].

The isomeric 1-, 2- and 4-phenylimidazoles are inhibitors of the oxidation of camphor by cytochrome P450<sub>cam</sub>. Nevertheless, only 1- and 4-phenylimidazole bind as expected, with one nitrogen atom of the imidazole ring coordinated to the iron atom of the porphyrin system; 2-phenylimidazole cannot be accommodated in such a position and binds in a completely different manner [37]. Structurally related dipeptide inhibitors of the serine protease elastase bind with their corresponding residues in different pockets of the enzyme [1,27,38]. The trypsin inhibitor *p*-guanidiniumbenzoate seems to be one of the rare cases where X-ray crystallography provides clear evidence that one and the same ligand binds in distinctly different orientations [39]. Many other examples of different binding modes of closely related analogs have been described in the literature [1,27–29]. From the observation of such differences, Dagmar Ringe proposed to use this information to design ‘hydra-headed’ inhibitors, which fill all possible pockets of a binding site.

## 6. Different Mechanisms of Action of Similar Molecules

Several well-known examples of different modes of action of closely related analogs can be found in medicinal chemistry and pharmacology textbooks. Norepinephrine **29a**, epinephrine **29b** and isoproterenol **29c** (Fig. 13) are adrenergic agonists. However, in going from R = H to R = CH<sub>3</sub> and R = isopropyl, the mechanism of action gradually changes from a more or less specific  $\alpha$ -adrenergic agonism to a pure  $\beta$ -adrenergic agonism. If the two hydroxyl groups of isoproterenol are changed into chloro substituents, the  $\beta$ -adrenergic antagonist dichloroisoproterenol (DCI) **30** results; in fact, **30** was the first  $\beta$ -blocker.

An unexpected effect resulted after the introduction of an isobutyl group into the AT<sub>1</sub>-specific angiotensin II receptor antagonist **31a** (Fig. 13). The original AT<sub>1</sub> selectivity is completely destroyed; in addition, the new analog **31b** is no longer an antagonist, it is a strong agonist at the AT<sub>1</sub> and AT<sub>2</sub> subtypes of this receptor [40]. A related effect has been observed for some structurally diverse cholecystokinin (CCK) receptor ligands; the introduction of an isopropyl group at a certain nitrogen atom leads from peripheral CCK antagonists to agonists [41,42].

The integrin family of receptors are structurally and functionally related membrane-imbedded glycoproteins that ‘integrate’ the extracellular matrix with the cytoskeleton.

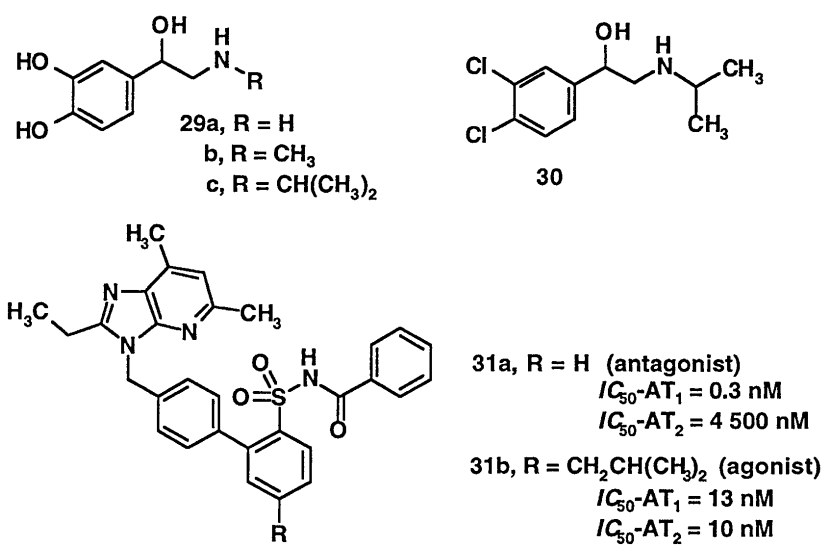


Fig. 13. The change from norepinephrine 29a to epinephrine 29b and isoproterenol 29c gradually changes the mode of action from  $\alpha$ -agonism to  $\beta$ -agonism. Dichloroisoproterenol (DCI) 30 was the first  $\beta$ -adrenergic antagonist. Introduction of an isobutyl group into the AT<sub>1</sub>-selective antagonist 31a produces the potent, unselective AT<sub>1</sub>/AT<sub>2</sub> agonist 31b.

44

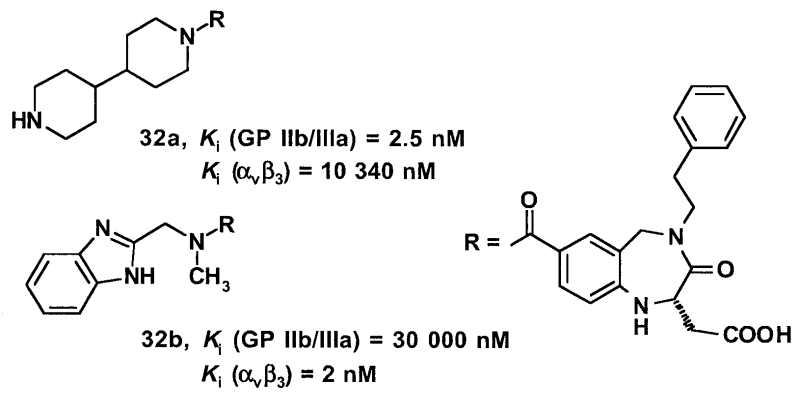


Fig. 14. The chemically closely related integrin antagonists **32a** and **32b** differ in their selectivity towards the fibrinogen (GPIIb/IIIa) and the vitronectin ( $\alpha_v\beta_3$ ) receptors by nearly eight orders of magnitude.

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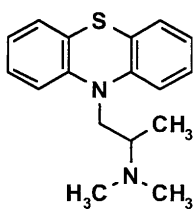
Important members are the GPIIb/IIIa receptor (GP for glycoprotein, IIb/IIIa for the  $\alpha_{IIb}\beta_3$  integrin) and the vitronectin ( $\alpha_v\beta_3$ ) receptor [43,44]. Whereas the GPIIb/IIIa receptor binds, *inter alia*, fibrinogen and mediates blood platelet aggregation, the  $\alpha_v\beta_3$  receptor binds vitronectin and is responsible for angiogenesis, vascular smooth muscle migration and adhesion of osteoclasts to the bone matrix. Thus, both receptors are important for drug design, the GP IIb/IIIa receptor for the development of antithrombotic agents and the vitronectin receptor for the development of drugs for the treatment of cancer, as well as against restenosis and osteoporosis. However, both fibrinogen and vitronectin interact with their receptors using an identical structural domain, the so-called RGD motif (RGD = Arg-Gly-Asp), but obviously in different conformations. This was first confirmed by the investigation of different cyclic pentapeptides, containing D-amino acids in different positions [44,45]. How far structure-based design (in this case, based only on the structures of ligands) can be advanced is illustrated by compounds **32a** and **32b**, which are highly selective GPIIb/IIIa and vitronectin receptor antagonists, respectively (Fig. 14) [46]. The selectivity of these analogs against the two receptors differs by nearly eight orders of magnitude, despite their close chemical similarity! The only major difference is the distance between the carboxylic group, which mimics the Asp, and the basic nitrogen atom, which mimics the Arg of the RGD motif.

A well-known example of different modes of action of closely related analogs are the anti-allergic agent promethazine **33**, the neuroleptic chlorpromazine **34**, and the antidepressants imipramine **35a** and desipramine **35b** (Fig. 15). Despite their very similar structures, **33** acts mainly as a histamine H1 antagonist, **34** is a dopamine antagonist, **35a** is an unspecific norepinephrine- and serotonin-uptake inhibitor and **35b** is a norepinephrine-specific uptake inhibitor. Steroid hormones — e.g. **36–39** (Fig. 16) — give another example of strikingly different biological effects of chemically closely related analogs.

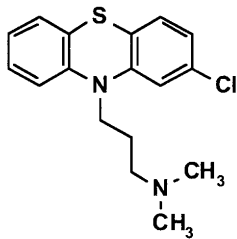
Several therapeutically used drugs bind to more than one receptor and are correspondingly termed ‘promiscuous’ ligands. However, whether a certain (balanced) unspecificity of their mode of action is advantageous for therapy or not still remains uncertain.

## 7. Chirality and Biological Activities

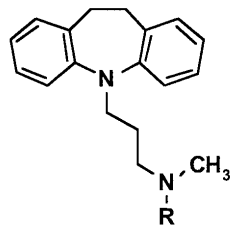
Due to the chiral nature of amino acids (except glycine), drug binding sites of proteins are asymmetric. In the past, the different actions of enantiomers of chiral molecules on enzymes and receptors were often neglected [47]. For economic reasons, racemates of synthetic drugs were used in therapy. Today, researchers and drug companies are more aware of the different effects of enantiomers and diastereomers [1,2] in their biological activities, as well as in their pharmacokinetics. Enantiomers can even be differentiated by their odor — e.g. the monoterpenes (*R*)- and (*S*)-limonene **40** and (*R*)- and (*S*)-carvone **41** (Fig. 17) [48]. Butaclamol **42** is mentioned as just one example of significantly different eudismic ratios (i.e. ratios of affinities of the different enantiomers) *versus* different receptors (Fig. 17) [47].



**33, Promethazine**  
(H<sub>1</sub> antagonist)



**34, Chlorpromazine**  
(D antagonist)



**35 a, R = CH<sub>3</sub>, Imipramine**  
**b, R = H, Desipramine**  
(uptake inhibitors)

*Fig. 15. Despite their chemical analogy, the tricyclic compounds 33, 34 and 35 have different mechanisms of action and different therapeutic effects; 33 is an antiallergic agent, 34 is used for the treatment of schizophrenia and 35a and b for the treatment of depression.*

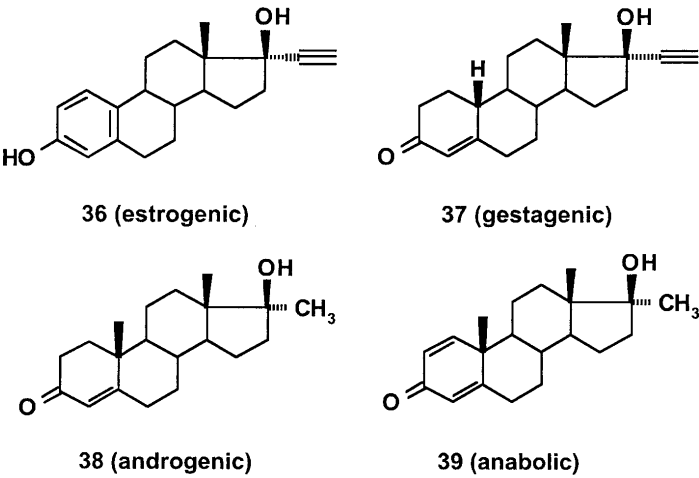
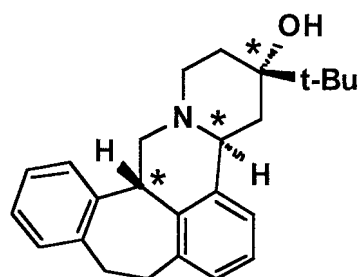
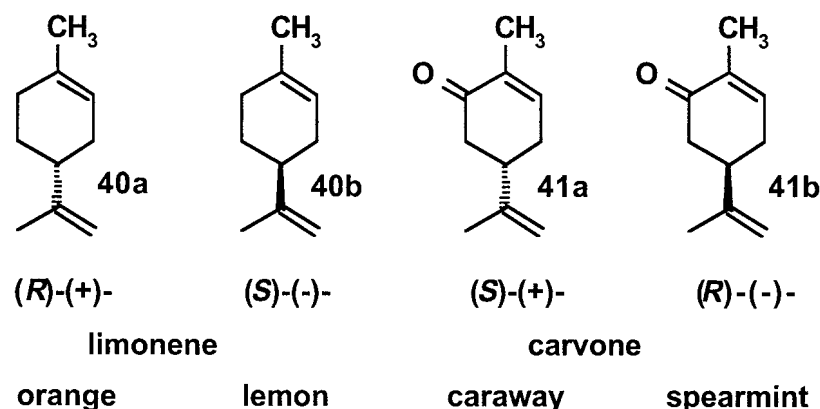


Fig. 16. In the steroid series, minor chemical differences produce very different effects on the biological properties of the compounds. Compounds 36–38 correspond in their biological actions to the different female and male sex hormones, compound 39 is a male sex hormone-related anabolic.



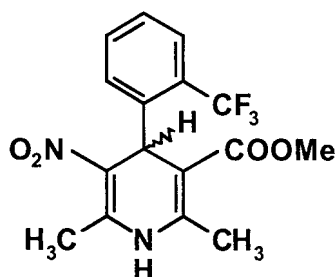
**42, (+)-enantiomer**

| Eudismic Ratios            |       |
|----------------------------|-------|
| $\alpha_1$ receptor        | 73    |
| D <sub>2</sub> receptor    | 1 250 |
| r-HT <sub>1</sub> receptor | 8     |
| 5-HT <sub>2</sub> receptor | 73    |
| muscarinic ACh receptor    | 0.5   |

Fig. 17. Enantiomers are different: the olfactory receptors of our nose can even distinguish such minor structural differences as between **40a** and **b**, as well as between **41a** and **b** (the typical odors of the compounds are indicated below the structures). Activity differences between enantiomers are defined by their eudismic indices, the ratios of biological activities of the individual enantiomers. They significantly depend on the biological target. Whereas both enantiomers of butaclamol **42** look very 'similar' to the muscarinic acetylcholine receptor, they differ by more than three orders of magnitude in their affinities to the D<sub>2</sub> receptor.

Enantiomers may even have opposite biological effects. Certain chiral barbiturates show sedative activities in the one enantiomer and convulsive activities in the other one. The nifedipine analog Bay K 8644, **43** (Fig. 18) was originally synthesized as a racemate. *In vitro* tests with isolated smooth muscle strips showed it to be more or less inactive. However, several years later its high affinity to calcium channels was discovered; this demanded a separation of the racemate into the two enantiomers. One is a calcium channel agonist, the other a weak antagonist. Höltje explained these differences by the asymmetry of the molecular electrostatic potentials of the enantiomers, if the molecules are superimposed by their ring systems [49].

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**43a, (S)-(-)-form: agonist**

**43b, (R)-(+)-form: weak antagonist**

Fig. 18. The enantiomers of Bay K 8644 have opposite biological effects. Whereas **43a** stabilizes the calcium channel in its open form, **43b** stabilizes its closed form.

## 8. The Similarity Principle in QSAR and 3D QSAR

Quantitative descriptions of the specific binding of ligands to a protein surface are based on the additivity principle of activity group contributions to the overall affinities. This has been extensively proven by dedicated investigations performed by Andrews, Goodford and Bohacek [50], but also by Böhm's scoring function of the *de novo* design program LUDI [51]. Some other approaches are reviewed in this book. However, despite the differences between enantiomers, discussed above, classical QSAR has no good tools to distinguish different conformers or enantiomers. 3D QSAR methods are much more efficient in this respect.

The very first approach to describe structure–activity relationships by  $N \times N$  similarity matrices ( $N$  = number of compounds) was presented by Rum and Herndon in 1991 [52]. More systematic investigations, using 3D similarity indices, were performed by Richards et al. in 1993 [53,54].  $N \times N$  Distance matrices are even appropriate for the quantitative description of nonlinear structure–activity relationships [50,55,56].

A very promising method, based on similarity fields, is the Comparative Molecular Similarity Indices Analysis (CoMSIA) approach [57,58]. As in CoMFA, all molecules are aligned according to a common pharmacophore; similarity indices of certain probes to the different molecules are then calculated in the positions of a regular grid. These fields, not just pairwise similarity indices of the molecules, are correlated with the biological activities. Due to the 'soft' character of the Gaussian potentials that are used to derive the similarity fields, smoother and more contiguous contour maps result from CoMSIA analyses [57].

As compared to the CoMFA and CoMSIA methods, the use of  $N \times N$  3D similarity matrices in QSAR has several advantages. Instead of a mutual alignment of all active and inactive molecules to a common reference frame, only pairwise alignments need to be performed [56,58]. This leads to a matrix with individual 'similarity vectors' for each analog. Inactive analogs do no longer distort the alignment of the active analogs. If SEAL similarity indices [59,60] are used in the generation of the  $N \times N$  matrix, not even a grid is needed. Thus, the frequently observed disturbance of the CoMFA results, after translation or rotation of the box around the molecules (e.g. [61]), are not ob-

served. A disadvantage of the calculation of pairwise similarity coefficients is the fact that no contour maps can be generated from the resulting QSAR model.

## 9. Conclusion

There are many lessons to be learned for 3D QSAR applications. Firstly, a correct alignment is the most important basis of each structure–activity analysis. Errors that are produced there can never lead to correct models.

An overreliance in target-independent similarity indices has to be questioned, because of the dependence of ‘similarity’ on the biological macromolecule to which the analogs bind. Sophisticated investigations on the ‘dissimilarity’ of chemical databases are most often futile. Similar compounds may have very different actions and different molecules can be very similar in their biological activities. Considering the examples presented in this chapter and the many more cases in the literature, one has to admit that we are far from a deeper understanding of the details which underline the observed structure–activity relationships. Applying the results from one series of analogs to another one may lead to completely wrong conclusions.

In the past, the use of molecular connectivity parameters in QSAR studies has led to highly controversial discussions. The same may happen to the BCUT, WHIM and EVA parameters, all described in other chapters in this volume. But one fact can be taken for sure: all these methods describe the similarity between molecules to a different extent. Thus, one should not be surprised that these approaches work, in some cases, even as well as other, more ‘rational’ methods.

Besides a correct alignment, the selection of the training and test sets is critically important to the results of a 3D QSAR study [58]. Only if both sets cover approximately the same range of structural space, can one be sure that the analysis and the results are meaningful. Cross-validation methods do not help in this respect. If the data set is highly redundant, even cross-validation in groups will produce ‘good’ results, whereas in clearly non-redundant datasets even a simple leave-one-out cross-validation must fail. One has to come back to the original goal of QSAR studies: to derive a working hypothesis and to design new analogs, based on one or several alternative working hypotheses. In this sense, QSAR is a theoretical tool which cannot solve all our problems but which definitely should accompany the experiments of the medicinal chemists and biologists.

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