

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

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



No. 08-030800- -00008-0000

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Señor

SUPERINTENDENTE DE INDUSTRIA Y COMERCIO

Superintendencia de Industria y Comercio

Ministerio de Desarrollo Económico

E. S. D.

RECURSO DE REPOSICION

Ref.: Expediente No. 08.030.800

Solicitud de Patente de Invención para:

**"DERIVADOS DE PIRIDIL-1,2,4-OXADIAZOL-5-ONA Y DERIVADOS DE FENIL-
[1,2,4]-OXADIAZOL-5-ONA CON GRUPO FENILO Y PROCEDIMIENTOS PARA
SU PREPARACIÓN"**

Solicitante: **SANOFI**

Yo, **MARGARITA CASTELLANOS ABONDANO**, mayor de edad, domiciliada e identificada como aparece al pie 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. **31823** de 25 de mayo de 2012, notificada por edicto desfijado el 29 de junio de 2012.

1. LA RESOLUCION No. 31823

La Superintendencia de Industria y Comercio expidió la Resolución No. 31823 por la cual se niega la patente que fue solicitada el día 28 de marzo de 2008, para el invento denominado: **"DERIVADOS DE PIRIDIL-1,2,4-OXADIAZOL-5-ONA Y DERIVADOS DE FENIL-[1,2,4]-OXADIAZOL-5-ONA CON GRUPO FENILO Y PROCEDIMIENTOS PARA SU PREPARACIÓN"**, y radicada bajo el expediente N° 08.030.800.



2. FUNDAMENTO DE LA RESOLUCION No. 31823

La Resolución 31823 establece en su artículo sexto que, las reivindicaciones 1 a 9, contenidas en los folios 283 y 285, 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° 31823 del 25 de mayo 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 del título y las reivindicaciones y el nivel inventivo frente a la combinación de las enseñanzas divulgadas por los documentos D1 (WO2005097762) y D2 (US5641796), 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 nivel inventivo frente a la combinación de las enseñanzas divulgadas por los documentos D1 (WO2005097762) y D2 (US5641796), sino frente a D1 y un nuevo documento D3 (WO2005097786).

Pongo de presente que dicho documento D3 no fue citado en la última acción oficial de fondo No. 130 notificada el 6 de enero de 2012, pero a partir de ese documento este Despacho concluyó que el “impedimento” relativo a nivel inventivo “subsiste”.

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 frente a la combinación de D1 y el nuevo documento del arte previo D3. No obstante, procedió a negar el privilegio de patente sobre la base de un nuevo “impedimento” surgido del estudio de fondo.

Es claro entonces que con la Resolución No. 31823 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 dicha 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. 31823 se vulneró el derecho de mi representado a conocer las nuevas 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, ya que habiendo citado el señor examinador 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. 130, la Administración no dio la oportunidad al solicitante de presentar sus argumentos y pruebas a favor del nivel inventivo frente al documento D3, el cual no fue mencionado en la acción oficial No. 130, 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. 31823 dando continuidad al trámite de la presente solicitud.

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

3.1 Incumplimiento del Artículo 18 de la Decisión 486

Este Despacho considera que las reivindicaciones 1 a 9 no cumplen el requisito del artículo 18 de la Decisión 486. Lo anterior por cuanto el documento D1 (WO2005097762) en combinación con D3 (WO2005097786) permitiría al técnico medio

versado en la materia técnica llegar a los compuestos reclamados en donde B se define como nitrógeno.

De acuerdo con la resolución No. 31823, el nuevo documento D3 citado por el examinador se considera el estado de la técnica más cercano a la invención definida por la reivindicación 1. Según el examinador, la única diferencia de la presente invención frente a D3, es que se tiene un anillo de piridina unido al anillo de oxadiazol-5-ona en lugar de un benceno, y que se tiene un radical R9. Sin embargo, *"no se evidencia que dicha diferencia genere algún efecto técnico, porque las actividades de los compuestos previamente revelados en el documento D3 son comparables con las de los compuestos de la presente solicitud"*

Por otro lado, según el examinador, *"el documento D1 divulga compuestos que pueden contener tanto un anillo de benceno como un anillo de piridina unido directamente al anillo de oxadiazol-5-ona conservándose la actividad como agonista de PPAR delta. Por tanto una persona de nivel medio versada en la materia se vería motivada a cambiar el anillo de benceno por un anillo de piridina con la expectativa razonable de conservar la actividad de los componentes revelados..."*. *"Por otro lado, respecto a los sustituyentes R9, no se evidencia que dicha sustitución genere algún efecto técnico inesperado sobre el grupo farmacofórico previamente revelado en el documento D3"*

Si embargo, contrario a lo afirmado por el examinador en la resolución 31823, la presente invención es inventiva frente a la combinación de las enseñanzas de D1 y D3.

El documento D3 se refiere a compuestos derivados de 1,2,4-oxadiazol-3-ona como agonistas de PPAR delta. Sin embargo, los compuestos de la presente invención presentan un grupo R9, el cual no puede ser en ningún caso hidrógeno, mientras que en D3, en la posición equivalente a R9, únicamente se permite la presencia de hidrógeno.

Por su parte, D1 divulga compuestos de 1,3,4-oxadiazol-2-onas que tienen actividad como moduladores de PPAR delta. Sin embargo, los compuestos aquí reclamados se refieren al grupo 1,2,4-oxadiazol-3-ona, y además poseen el sustituyente R9, el cual en D1 sólo puede corresponder a hidrógeno.

En vista de lo anterior, ninguno de los documentos D1 o D3 divulga o sugiere otro sustituyente diferente del hidrógeno en la posición correspondiente a R9 de la presente

invención. Por lo tanto, ni D1 ni D3 proporciona sugerencia alguna para modificar los compuesto de D3 para introducir el sustituyente específico R9 como se encuentra aquí reclamado, en vez de un átomo de hidrógeno.

Adicionalmente, ni D1 ni D3 presenta las enseñanzas o sugerencias de que dichos compuestos de la presente invención con R9 puedan ser apropiados como agonistas de PPAR delta y PRAR alfa.

De este modo, traigo a colación la concesión de la solicitud equivalente US 7.834.030 que contiene reivindicaciones idénticas a las de la publicación internacional WO 2007/037178, de la cual la presente invención es fase nacional.

Adicionalmente, presento un pliego reivindicatorio modificado de 10 reivindicaciones, en el cual se ha incluido la reivindicación 8 que menciona los compuestos específicos de acuerdo con la reivindicación 1, junto con el correspondiente comprobante de pago **12-0053861** del 29 de mayo de 2012.

Con el fin de mostrar que no es posible afirmar que los compuestos de la presente invención por tener características estructurales 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 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.

Habiendo quedado sustentado el nivel inventivo de la solicitud, solicito a su Despacho que revoque la resolución 31823 y se otorgue el privilegio de patente a la invención titulada **“DERIVADOS DE PIRIDIL-1,2,4-OXADIAZOL-5-ONA Y DERIVADOS DE FENIL-[1,2,4]-OXADIAZOL-5-ONA CON GRUPO FENILO Y PROCEDIMIENTOS PARA SU PREPARACIÓN”**

De considerar que las nuevas objeciones presentadas por el examinador en dicha resolución denegatoria no son superadas en su totalidad, solicitamos muy comedidamente se continúe el trámite de la presente solicitud, con la emisión de una acción oficial adicional.

4. FUNDAMENTO 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. **31823** del 25 de mayo 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 **“DERIVADOS DE PIRIDIL-1,2,4-OXADIAZOL-5-ONA Y DERIVADOS DE FENIL-[1,2,4]-OXADIAZOL-5-ONA CON GRUPO FENILO Y PROCEDIMIENTOS PARA SU PREPARACIÓN”**

6. PRUEBAS

- Solicito que se tengan como tales, los documentos contenidos en el Expediente No. **08.030.800** de la referencia, en el que se presentó la solicitud de privilegio de patente para la invención titulada "**DERIVADOS DE PIRIDIL-1,2,4-OXADIAZOL-5-ONA Y DERIVADOS DE FENIL-[1,2,4]-OXADIAZOL-5-ONA CON GRUPO FENILO Y PROCEDIMIENTOS PARA SU PREPARACIÓN**" a nombre de la sociedad **SANOFI**.

7. NOMBRE Y DIRECCION DEL RECURRENTE

SANOFI

174 Avenue de France

75013 Paris

Francia

8. NOTIFICACIONES

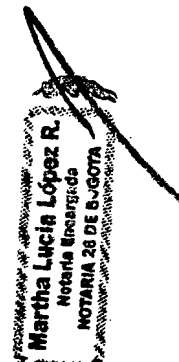
Las recibiré en la Secretaría de su Despacho y en mí oficina en la **Calle 94 A No. 7 A-09**, teléfono 7560770.



Del Señor Superintendente, Atentamente,

Margarita Castellanos Abondano
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. 09/11/2002



El anterior memorial fue presentado personalmente por

Margarita Caballero Abondano

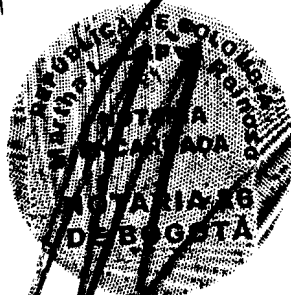
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de Usaquen

Tarjeta Profesional No. 70819 C.T.

Bogotá, D. E. - 9 III. 2012

Margarita Caballero Abondano



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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
NIT : 800.176.089-2
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Industria y Comercio SUPERINTENDENCIA	RECIBO DE CAJA	No. 12 - 0053861
		Bogotá D.C., Mayo 29 de 2012 - 14:14:41

RECIBIDO DE : CASTELLANOS Y Y CO	NI 800.174.641
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*** Soporte del Pago ***					
TIPO PAGO	BANCO	CUENTA	No. PAGO	FECHA PAGO	VR. PAGO
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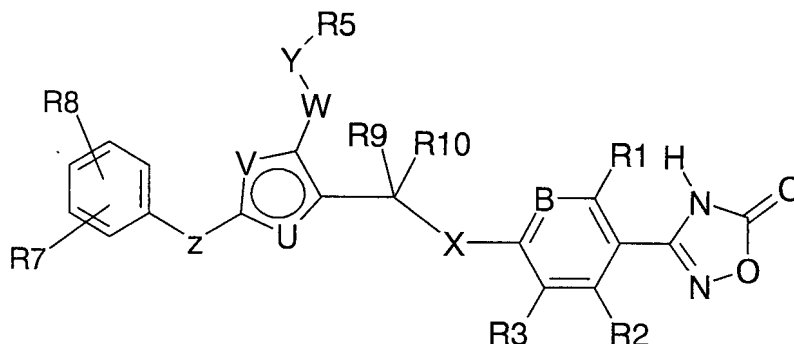
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1	50005-01-03 CAMBIO DE MODALIDAD Y MODIFICACIONES	EN CREACION CORRECCION A SOLICITUD EN TRAMITE / MODIFICA	120.000.00 120.000.00
			\$120.000.00

SON: **CIENTO VEINTE MIL PESOS MONEDA CORRIENTE***

Responsable: _____
Recibo de Caja Aplicado al Expediente No. _____

REIVINDICACIONES MODIFICADAS RECURSO

1. Compuestos de fórmula I:



fórmula I

en la que

R1 es H, halógeno, alquilo (C1-C4), alquilen (C0-C4)-O-alquilen (C0-C2)-H, cicloalquilo (C3-C6), fenilo, en el que alquilo, alquilen y fenilo no están sustituidos o están mono, di- o tri-sustituidos con F;

B es C(R4) o N;

R2 es H y

R3 es H o halógeno;

R2 y R3 junto con los átomos de carbono a los que están unidos forman un anillo (C6) o heteroarilo (C5-C6), en donde uno o más átomos de carbono está reemplazado por un heteroátomo seleccionado de N, O y S;

R4 es H,

X es O, CH₂, O-CH₂;

Z es un enlace;

V es N;

U es O, S;

W es un enlace, alquilen (C1-C3);

Y es un enlace, N(R6);

R5 es H,

R5 y R6 junto con el átomo de nitrógeno al que están unidos ($Y = N(R6)$) forman una piperidina que está monosustituida por CF_3 ;

R7 es CF_3 ;

R8 es H;

R9 es alquilo (C1-C4), alquilen (C0-C3)-fenilo, alquilen (C0-C3)-heteroarilo (C5-C6), en el que uno o más átomos de carbono se reemplazan por heteroátomos seleccionados de N, O y S; alquilen (C0-C3) – cicloalquilo (C3-C6), en donde alquilo, alquilen, fenilo y heteroarilo no están sustituidos o están mono, di o tri-sustituidos con F;

R10 es H;

en todas sus formas estereoisómeras, formas enantiómeras y mezclas en cualquier relación, y sus sales farmacéuticamente aceptables y formas tautoméricas.

2. Compuestos de la fórmula I según la reivindicación 1, en los que

B es $C(R_4)$ y

R4 es H.

3. Compuestos de fórmula I según las reivindicaciones 1 ó 2, en los que

X es O, O-CH₂.

4. Compuestos de la fórmula I según las reivindicaciones 1 a 3, en los que

R2 es H y

R3 es H, F

ó

R2 y R3 junto con los átomos de carbono a los que están unidos forman un anillo arilo (C6) o heteroarilo (C5-C6), en donde uno o más átomos de carbono se reemplazan por heteroátomos seleccionados de N, O y S.

5. Compuestos de la fórmula I según las reivindicaciones 1 a 4, en los que

W es CH₂,

Y es un enlace,

R5 es H.

6. Compuestos de la fórmula I según las reivindicaciones 1 a 5, en los que

U es S y

V es N.

7. Compuestos de la fórmula I según las reivindicaciones 1 o 3 a 6, en donde uno o más sustituyentes tienen los siguientes significados:

B es C(R₄), N;

R₁ es H, F, Cl, Br, OH, O-CH₃, O-CHF₂, O-CH₂-CF₃, CF₃, CH₂-CH₃, CH₂-O-CH₂-CF₃, CH₂-O-CH₃, CH₂-O-CH₂-CH₃; ciclopropilo;

R₂ es H;

R₃ es H, F;

R₄ es H;

R₂ y R₃ junto con los átomos de C a los que están unidos y el anillo que los tiene forman un naftaleno o un anillo quinolina.

X es O, CH₂, O-CH₂;

V es N,

U es S;

W es CH₂;

Y es un enlace, N(R₆);

R₅ es H;

R₅ y R₆, junto con el átomo de nitrógeno al que están unidos (Y = N(R₆)), forman una piperidina que está monosustituída con CF₃;

Z es un enlace;

R₇ es CF₃;

R₈ es H;

R₉ es CH₃, CH₂CH₃, C₃H₇, C₄H₉, CF₃, CF₂-CH₂-CH₃, fenilo, CH₂-fenilo, CH₂-CH₂-fenilo, CH₂-4-F-fenilo, CH-piridilo, CF₂-ciclopropilo, CF₂-4-CHF₂-fenilo;

R₁₀ es H.

8. Los compuestos de fórmula I de acuerdo con la reivindicación 1, seleccionados entre la siguiente serie:

3-(2-Cloro-4-{1-[4-metil-2-(4-trifluorometilfenil)-tiazol-5-il]etoxi}fenil)-4H-[1,2,4]-oxadiazol-5-ona

3-(2-Cloro-4-{2-metil-1-[4-metil-2-(4-trifluorometilfenil)tiazol-5-il]propoxi}fenil)-4H-[1,2,4]-oxadiazol-5-ona

3-(2-Cloro-4-{3-metil-1-[4-metil-2-(4-trifluorometilfenil)tiazol-5-il]butoxi}fenil)-4H-[1,2,4]-oxadiazol-5-ona

3-(2-Cloro-4-{1-[4-metil-2-(4-trifluorometilfenil)tiazol-5-il]propoxi}fenil)-4H-[1,2,4]-oxadiazol-5-ona

3-(2-Cloro-4-{1-[4-metil-2-(4-trifluorometilfenil)tiazol-5-il]-2-feniletoksi}fenil)-4H-[1,2,4]-oxadiazol-5-ona

3-(2-Cloro-4-{[4-metil-2-(4-trifluorometilfenil)tiazol-5-il]fenilmetoxi}fenil)-4H-[1,2,4]-oxadiazol-5-ona

3-(2-Cloro-4-{1-[4-metil-2-(4-trifluorometilfenil)tiazol-5-il]-3-fenilpropoxi}fenil)-4H-[1,2,4]-oxadiazol-5-ona

3-(2-Cloro-4-{1-[4-metil-2-(4-trifluorometilfenil)oxazol-5-il]-3-fenilpropoxi}fenil)-4H-[1,2,4]-oxadiazol-5-ona

3-(2-Cloro-4-{2-(4-fluorfenil)-1-[4-metil-2-(4-trifluorometilfenil)tiazol-5-il]etoxi}fenil)-4H-[1,2,4]-oxadiazol-5-ona

3-(2-Cloro-4-{1-[4-metil-2-(4-trifluorometilfenil)tiazol-5-il]2-pyridin-2-iletoksi}fenil)-4H-[1,2,4]-oxadiazol-5-ona

3-(2-Bromo-4-{1-[4-metil-2-(4-trifluorometilfenil)tiazol-5-il]propoxi}fenil)-4H-[1,2,4]-oxadiazol-5-ona

3-[4-{1-[4-Metil-2-(4-trifluorometilfenil)tiazol-5-il]propoxi}2-(2,2,2-trifluoroetoximetil)fenil]-4H-[1,2,4]-oxadiazol-5-ona

3-(2-Metoximetil-4-{1-[4-metil-2-(4-trifluorometilfenil)tiazol-5-il]propoxi}fenil)-4H-[1,2,4]-oxadiazol-5-ona

3-(2-Etoximetil-4-{1-[4-metil-2-(4-trifluorometilfenil)tiazol-5-il]propoxi}fenil)-4H-[1,2,4]-oxadiazol-5-ona

3-(2-Etil-4-{1-[4-metil-2-(4-trifluorometilfenil) tiazol-5-il]propoxi}fenil)-4H-[1,2,4]-oxadiazol-5-ona

3-(2-Cyclopropil-4-{1-[4-metil-2-(4-trifluorometilfenil) tiazol-5-il]propoxi}fenil)-4H-[1,2,4]-oxadiazol-5-ona

3-(4-{1-[4-Metil-2-(4-trifluorometilfenil) tiazol-5-il]propoxi}naftalen-1-il)-4H-[1,2,4]-oxadiazol-5-ona

3-(4-{1-[4-Metil-2-(4-trifluorometilfenil) tiazol-5-il]propoxi}2-trifluorometilfenil)-4H-[1,2,4]-oxadiazol-5-ona

3-(4'-Fluorò-5-{1-[4-metil-2-(4-trifluorometilfenil) tiazol-5-il]propoxi}bifenil-2-il)-4H-[1,2,4]-oxadiazol-5-ona

3-(2-Cloro-4-{2,2,2-trifluoro-1-[4-metil-2-(4-trifluorometilfenil) tiazol-5-il]etoxi}fenil)-4H-[1,2,4]-oxadiazol-5-ona

3-(2-Cloro-4-{2,2-difluoro-1-[4-metil-2-(4-trifluorometilfenil) tiazol-5-il]butoxi}fenil)-4H-[1,2,4]-oxadiazol-5-ona

3-(2-Fluoro-4-{2,2,2-trifluoro-1-[4-metil-2-(4-trifluorometilfenil) tiazol-5-il]etoximetil}fenil)-4H-[1,2,4]-oxadiazol-5-ona

3-(2-Cloro-4-{2,2,2-trifluoro-1-[4-metil-2-(4-trifluorometilfenil) tiazol-5-il]-etoximetil}fenil)-4H-[1,2,4]-oxadiazol-5-ona

3-(2-Cyclopropil-4-{2,2,2-trifluoro-1-[4-metil-2-(4-trifluorometilfenil) tiazol-5-il]etoximetil}fenil)-4H-[1,2,4]-oxadiazol-5-ona

3-(8-{2,2,2-Trifluoro-1-[4-metil-2-(4-trifluorometilfenil) tiazol-5-il]etoximetil}quinolin-5-il)-4H-[1,2,4]-oxadiazol-5-ona

3-(4-{2,2,2-Trifluoro-1-[4-metil-2-(4-trifluorometilfenil) tiazol-5-il]etoximetil}naftalen-1-il)-4H-[1,2,4]-oxadiazol-5-ona

3-(2-Fluoro-4-{1-[4-metil-2-(4-trifluorometilfenil)tiazol-5-il]propoximetil}fenil)-4H-[1,2,4]-oxadiazol-5-ona

3-(4-{1-[4-Metil-2-(4-trifluorometilfenil)tiazol-5-il]propoximetil}naftalen-1-il)-4H-[1,2,4]-oxadiazol-5-ona

3-(4-{1-[4-Metil-2-(4-trifluorometilfenil)tiazol-5-il]propoximetil}fenil)-4H-[1,2,4]-oxadiazol-5-ona

3-(4-{2,2-Difluoro-1-[4-metil-2-(4-trifluorometilfenil)tiazol-5-il]butoximetil}fenil)-4H-[1,2,4]-oxadiazol-5-ona

3-(4-{2-Cyclopropil-2,2-difluoro-1-[4-metil-2-(4-trifluorometilfenil)tiazol-5-il]etoximetil}fenil)-4H-[1,2,4]-oxadiazol-5-ona

3-(4-{2-(4-Difluorometilfenil)-2,2-difluoro-1-[4-metil-2-(4-trifluorometilfenil)tiazol-5-il]etoximetil}fenil)-4H-[1,2,4]-oxadiazol-5-ona

3-(2-Fluoro-4-{2-[4-metil-2-(4-trifluorometilfenil)tiazol-5-il]hexil}fenil)-4H-[1,2,4]-oxadiazol-5-ona

3-(2-Fluoro-4-{1-[4-metil-2-(4-trifluorometilfenil)tiazol-5-il]propoxi}fenil)-4H-1,2,4-oxadiazol-5-ona

3-(2-Fluoro-4-((R)-1[2-(4-trifluorometilfenil)-4-(4-trifluorometilpiperidin-1-ilmetil)tiazol-5-il]propoxi}fenil)-4H-1,2,4-oxadiazol-5-ona

3-(2-Difluorometoxi-4-{1-[4-metil-2-(4-trifluorometilfenil)tiazol-5-il]propoxi}fenil)-4H-1,2,4-oxadiazol-5-ona

3-(2-Metoxi-4-{1-[4-metil-2-(4-trifluorometilfenil)-tiazol-5-il]propoxi}fenil)-4H-1,2,4-oxadiazol-5-ona

3-(2-Metoxi-4-((R)-1-[4-metil-2-(4-trifluorometilfenil)tiazol-5-il]propoxi}fenil)-4H-1,2,4-oxadiazol-5-ona

3-(2-Hydroxi-4-{1-[4-metil-2-(4-trifluorometilfenil)tiazol-5-il]propoxi}fenil)-4H-1,2,4-oxadiazol-5-ona

3-(5-Fluoro-2-metoxi-4-{1-[4-metil-2-(4-trifluorometilfenil)tiazol-5-il]propoxi}fenil)-4H-1,2,4-oxadiazol-5-ona

3-(5-Fluoro-2-metoxi-4-((R)-1-[4-metil-2-(4-trifluorometilfenil)tiazol-5-il]propoxi}fenil)-4H-1,2,4-oxadiazol-5-ona

3-(2-Difluorometoxi-5-fluoro-4-((R)-1-[4-metil-2-(4-trifluorometilfenil)tiazol-5-il]propoxi}fenil)-4H-1,2,4-oxadiazol-5-ona

3-(2-Metoxi-4-{1-[2-(4-trifluorometilfenil)-4-(4-trifluorometilpiperidin-1-ilmetil)tiazol-5-il]propoxi}fenil)-4H-1,2,4-oxadiazol-5-ona

3-(5-Fluoro-2-metoxi-4-{1-[2-(4-trifluorometilfenil)-4-(4-trifluorometilpiperidin-1-ilmetil)tiazol-5-il]propoxi}fenil)-4H-1,2,4-oxadiazol-5-ona

3-(2-(2,2,2-Trifluoroetoxi)-4-{1-[2-(4-trifluorometilfenil)-4-(4-trifluorometilpiperidin-1-ilmetil)tiazol-5-il]propoxi}fenil)-4H-1,2,4-oxadiazol-5-ona

3-(2-Difluorometoxi-4-{1-[2-(4-trifluorometilfenil)-4-(4-trifluorometilpiperidin-1-ilmetil)tiazol-5-il]propoxi}fenil)-4H-1,2,4-oxadiazol-5-ona

3-(2-Difluorometoxi-4-((R)-1-[2-(4-trifluorometilfenil)-4-(4-trifluorometilpiperidin-1-ilmetil)tiazol-5-il]propoxi}fenil)-4H-1,2,4-oxadiazol-5-ona

3-(2-Difluorometoxi-5-fluoro-4-{1-[2-(4-trifluorometilfenil)-4-(4-trifluorometilpiperidin-1-ilmetil)tiazol-5-il]propoxi}fenil)-4H-1,2,4-oxadiazol-5-ona

3-[5-Fluoro-4-{1-[4-metil-2-(4-trifluorometilfenil)tiazol-5-il]propoxi}-2-(2,2,2-trifluoroetoxi)fenil]-4H-1,2,4-oxadiazol-5-ona

3-[4-{1-[4-Metil-2-(4-trifluorometilfenil)tiazol-5-il]propoxi}-2-(2,2,2-trifluoroetoxi)fenil]-4H-1,2,4-oxadiazol-5-ona

3-[4-{(R)-1-[4-Metil-2-(4-trifluorometilfenil)tiazol-5-il]propoxi}-2-(2,2,2-trifluoroetoxi)fenil]-4H-1,2,4-oxadiazol-5-ona

3-(5-Fluoro-2-(2,2,2-trifluoroetoxi)-4-{1-[2-(4-trifluorometilfenil)-4-(4-trifluorometilpiperidin-1-ilmetil)tiazol-5-il]propoxi}fenil)-4H-1,2,4-oxadiazol-5-ona.

9 Una composición farmacéutica que comprende uno o más compuestos de fórmula I, según se reivindica en una o más de las reivindicaciones 1 a 8

10. Un procedimiento para preparar un producto farmacéutico que comprende uno o más de los compuestos según una o más de las reivindicaciones 1 a 8, que comprende mezclar el compuesto activo con un vehículo farmacéuticamente aceptable y poner esta mezcla en una forma de administración adecuada.



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Similarity and Dissimilarity: A Medicinal Chemist's View

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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 bioisoteric 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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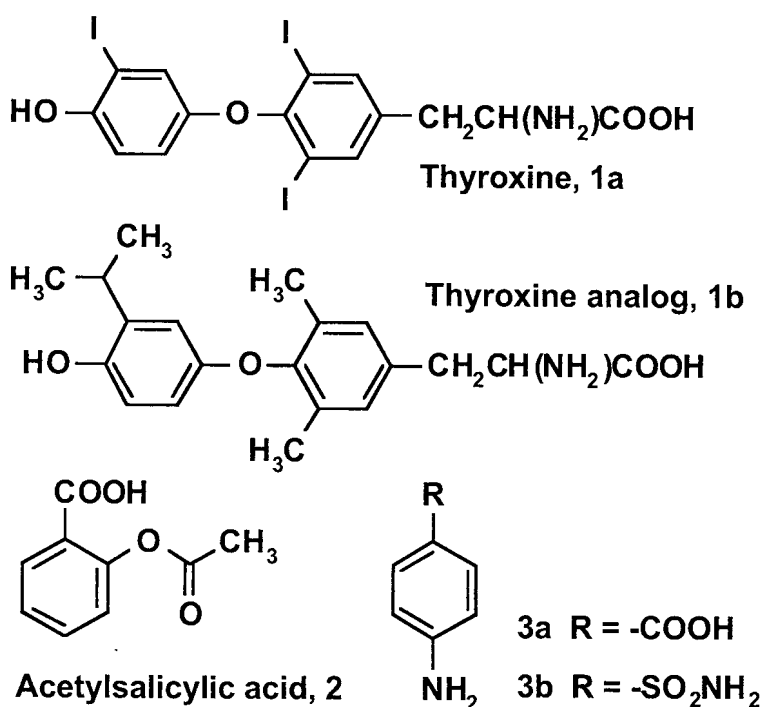


Fig. 1. Different effects of isosteric replacement. (a) The substitution of all three iodine atoms of thyroxine 1a leads to an analog 1b which still acts as a thyromimetic agent. (b) Replacement of an ester function, -O-CO-, by an amide function, -NH-CO-, most often produces analogs with higher metabolic 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, -SO₂NH₂, 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-

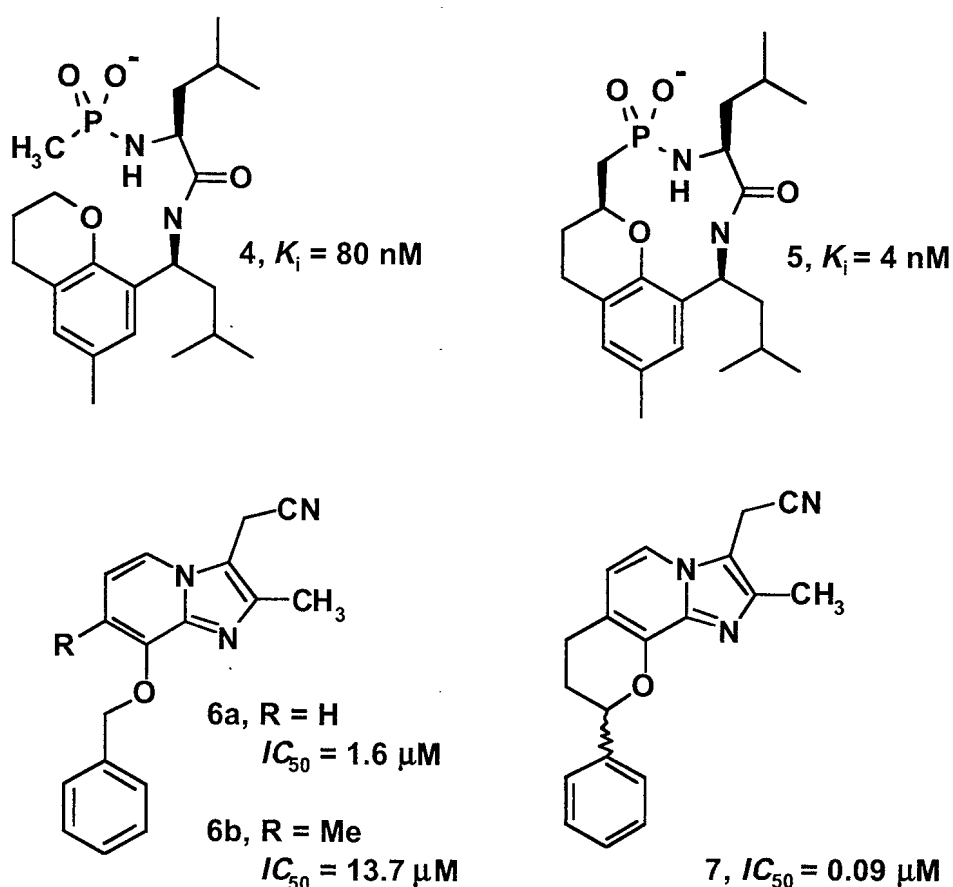


Fig. 2. Rigidification of a bioactive conformation significantly increases biological activities. (a) If an additional ring is introduced into the thermolysin inhibitor 4 to produce analog 5, a 20-fold increase in affinity is observed. (b) Introduction of a methyl group into the H^+/K^+ -ATPase inhibitor 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_α - C_β 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.

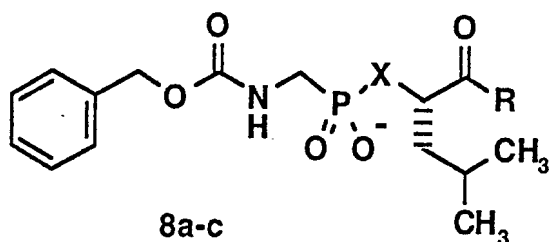
A lead structure, where a significant increase in binding affinity is achieved by steric constraints, not by ring closure, has been described by Kaplan and Bartlett [9]. In the flexible phosphonate tripeptide Cbz-Phe-Ala[PO_2^-]-O-CH(CH₃)COOH (= Z-Phe-Ala^P-(O)-Ala; ^P indicates a PO_2^- residue instead 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^P-(O)-Ala, Z-Phe-Ala^P-(O)-Phe and Z-Phe-Val^P-(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 –CH₂–, 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 –CH₂– 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 –CH₂– (**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 μM

R	X =	8a -NH-	8b -O-	8c -CH ₂ -
-OH		0.76	660	1.4
-Gly-OH		0.27	230	0.3
-Leu-OH		0.01	9	0.01

Fig. 3. The $X = -\text{NH}-$ 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 $-\text{O}-$ analogs **8b**, the $-\text{CH}_2-$ analogs **8c** cannot form the hydrogen-bond to Ala 113, but this disadvantage is counterbalanced by the effect that no desolvation of the CH_2 group of the ligand and no electrostatic repulsion take place. The differences between $X = -\text{NH}-$, $-\text{O}-$ and $-\text{CH}_2-$ are identically observed whether $R = -\text{OH}$, $-\text{Gly}-\text{OH}$ or $-\text{Leu}-\text{OH}$.

structure-activity relationship, as compared to the preceeding examples. Here the lipophilic $-\text{CH}_2-$ and $-\text{S}-$ analogs are much less active than the $-\text{NH}-$ and $-\text{O}-$ 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 $X = -\text{NH}-$ should form a hydrogen bond with one of the Asp 226 oxygen atoms. Thus, the replacement of $-\text{NH}-$ by $-\text{O}-$ 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]'.

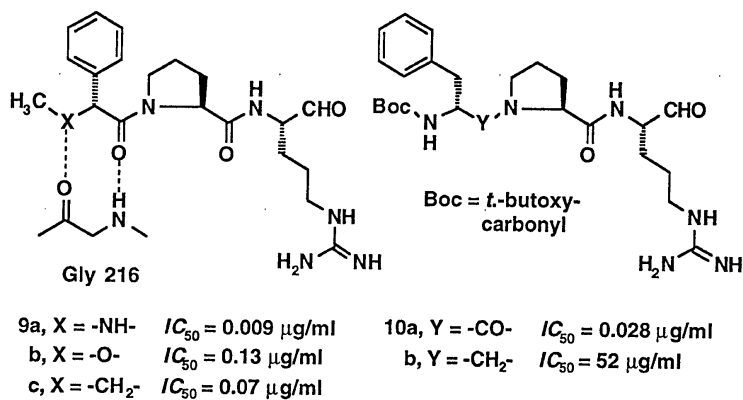


Fig. 4. The inhibitor 9a forms two hydrogen-bonds with glycine 216 of the serine protease thrombin. As compared to the thermolysin inhibitors 8a-c (Fig. 3), a similar but much less pronounced effect is observed by structural variation of the group X in the thrombin inhibitors 9a-c. If, on the other hand,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

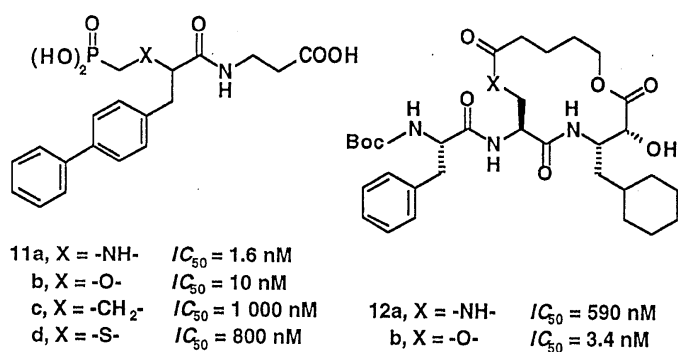
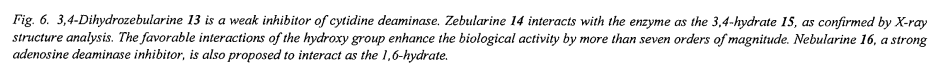


Fig. 5. The NEP 24.11 inhibitors 11a and b are much more potent than the more lipophilic analogs 11c and d. The macrocyclic renin inhibitor 12a is less potent than its oxygen analog 12b, although its NH group should form a favorable hydrogen-bond with the carboxylate of Asp 226 of the enzyme.



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 β -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

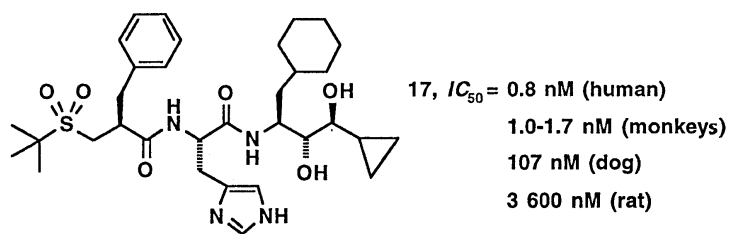
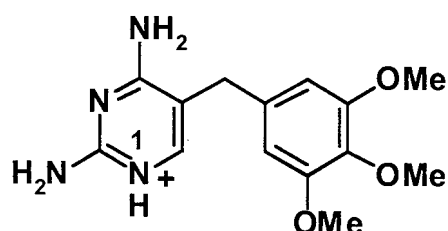


Fig. 7. Remikiren 17 is a much stronger inhibitor versus human and monkey renins, as compared to dog and rat renins. If this compound had only been tested in small laboratory animals (as was the case before gene technology could be applied in drug research), it would not have been considered for further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

n = 1

21b

R = α -H

n = 0

21c

R = β -H

n = 0

	IC_{50} values		
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 α -H to β -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].

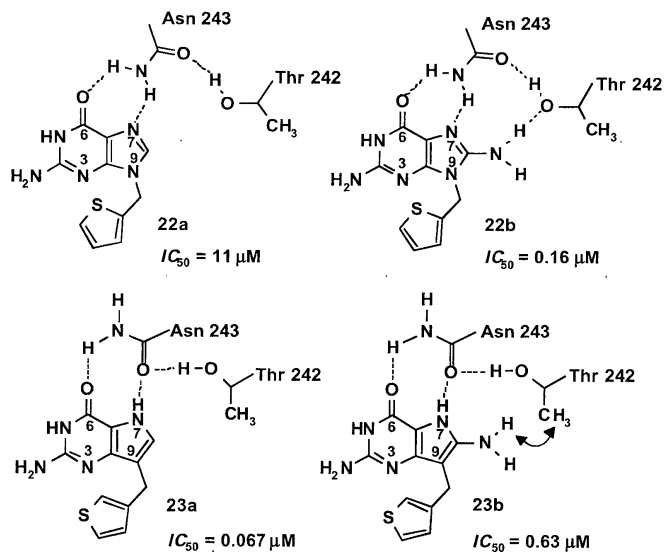
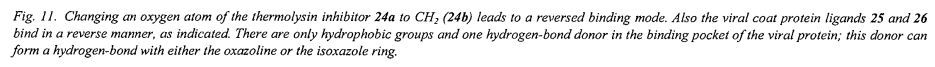


Fig. 10. The X-ray structures of the purine nucleoside phosphorylase (PNP) complexes with the inhibitors 22 and 23 show that 22a forms less favorable hydrogen-bonds with Asp 243 than the desazapurine 23a. On the other hand, the introduction of an 8-amino group has different consequences in 22a and 23a.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



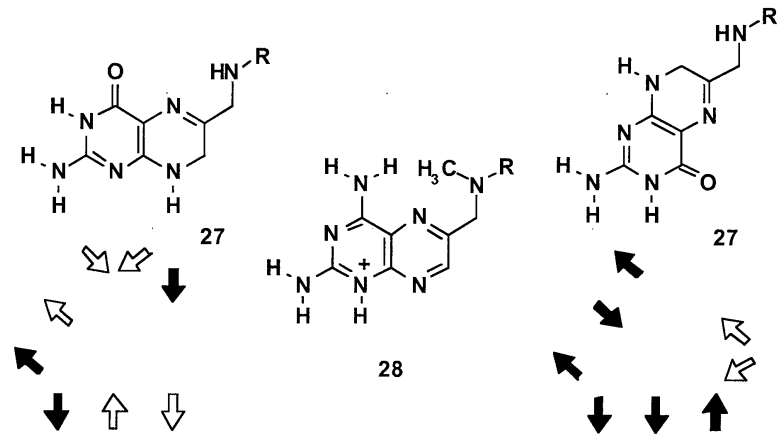


Fig. 12. Dihydrofolate 27 and the positively charged form of methotrexate 28 ($R = p\text{-C}_6\text{H}_4\text{-CONHCH}(\text{COOH})\text{CH}_2\text{CH}_2\text{COOH}$) have different patterns of hydrogen-bond donors and acceptors, if they are superimposed according to their ring systems (left side). 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 β -phenylpropionyl-phenylalanine (β -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_{cam}. 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 = CH₃ and R = isopropyl, the mechanism of action gradually changes from a more or less specific α -adrenergic agonism to a pure β -adrenergic agonism. If the two hydroxyl groups of isoproterenol are changed into chloro substituents, the β -adrenergic antagonist dichloroisoproterenol (DCI) **30** results; in fact, **30** was the first β -blocker.

An unexpected effect resulted after the introduction of an isobutyl group into the AT₁-specific angiotensin II receptor antagonist **31a** (Fig. 13). The original AT₁ selectivity is completely destroyed; in addition, the new analog **31b** is no longer an antagonist, it is a strong agonist at the AT₁ and AT₂ 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.

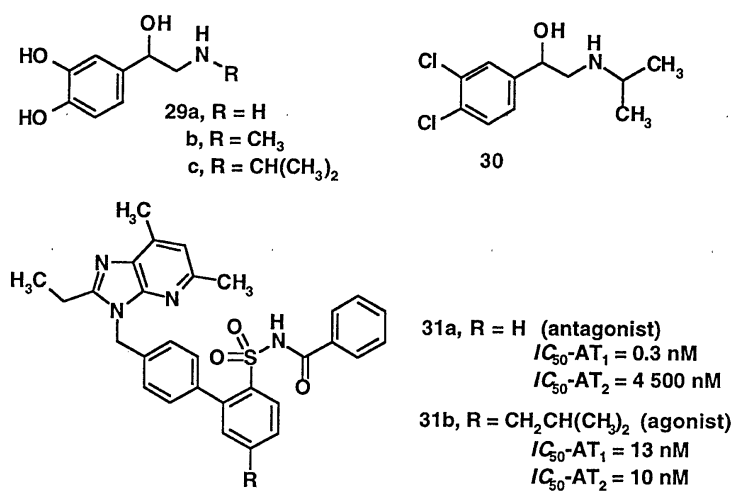


Fig. 13. The change from norepinephrine 29a to epinephrine 29b and isoproterenol 29c gradually changes the mode of action from α-agonism to β-agonism. Dichloroisoproterenol (DCI) 30 was the first β-adrenergic antagonist. Introduction of an isobutyl group into the AT₁-selective antagonist 31a produces the potent, unselective AT₁/AT₂ agonist 31b.

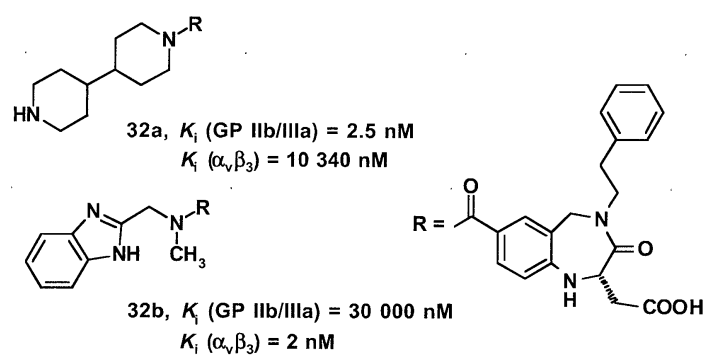


Fig. 14. The chemically closely related integrin antagonists 32a and 32b differ in their selectivity towards the fibrinogen (GP IIb/IIIa) and the vitronectin ($\alpha_v\beta_3$) receptors by nearly eight orders of magnitude.

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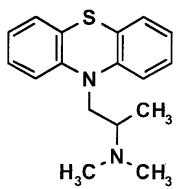
Important members are the GPIIb/IIIa receptor (GP for glycoprotein, IIb/IIIa for the $\alpha_{IIb}\beta_3$ integrin) and the vitronectin ($\alpha_v\beta_3$) receptor [43,44]. Whereas the GPIIb/IIIa receptor binds, *inter alia*, fibrinogen and mediates blood platelet aggregation, the $\alpha_v\beta_3$ 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 anti-depressants 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.

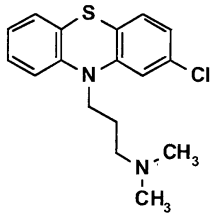
Several therapeutically used drugs bind to more than one receptor and are correspondingly termed ‘promiscuous’ ligands. However, whether a certain (balanced) unspecificity of their mode of action is advantageous for therapy or not still remains uncertain.

7. Chirality and Biological Activities

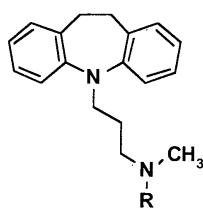
Due to the chiral nature of amino acids (except glycine), drug binding sites of proteins are asymmetric. In the past, the different actions of enantiomers of chiral molecules on enzymes and receptors were often neglected [47]. For economic reasons, racemates of synthetic drugs were used in therapy. Today, 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
(H₁ antagonist)



34, Chlorpromazine
(D antagonist)



35 a, R = CH₃, 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.

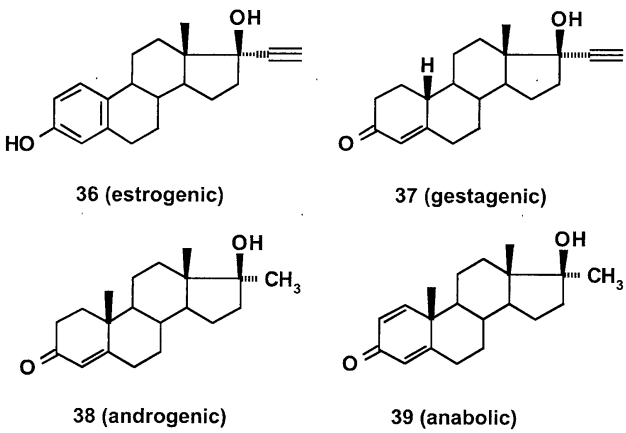


Fig. 16. In the steroid series, minor chemical differences produce very different effects on the biological properties of the compounds. Compounds 36–38 correspond in their biological actions to the different female and male sex hormones, compound 39 is a male sex hormone-related anabolic.

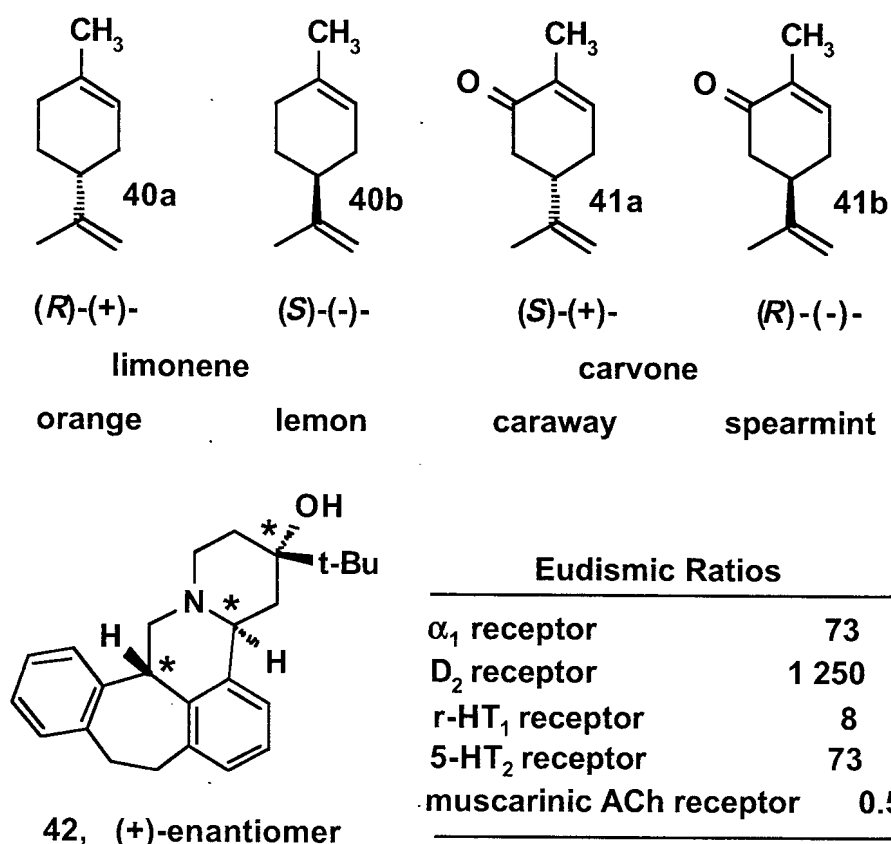


Fig. 17. Enantiomers are different: the olfactory receptors of our nose can even distinguish such minor structural differences as between 40a and b, as well as between 41a and b (the typical odors of the compounds are indicated below the structures). Activity differences between enantiomers are defined 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 D₂ 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. Hölftje 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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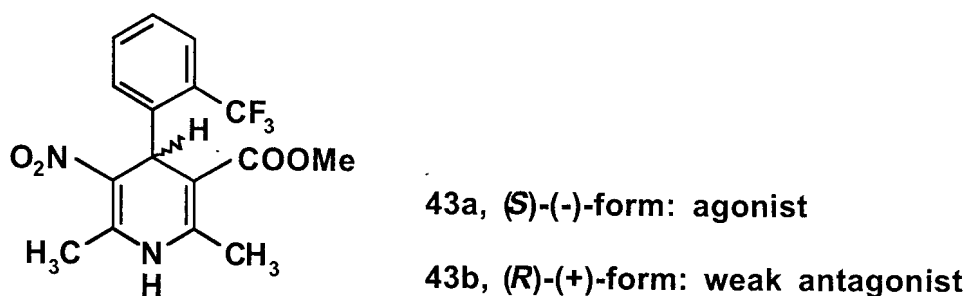


Fig. 18. The enantiomers of Bay K 8644 have opposite biological effects. Whereas 43a stabilizes the calcium channel in its open form, 43b stabilizes its closed form.

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.

References

1. Böhm, H.-J., Klebe, G. and Kubinyi, H., *Wirkstoffdesign. Der Weg zum Arzneimittel*, Spektrum Akademischer Verlag, Heidelberg, 1996.
2. Wermuth, C.G. (Ed.), *The Practice of Medicinal Chemistry*, Academic Press, London, 1996.
3. Wolff, M.E. (Ed.), *Burger's Medicinal Chemistry*, 5th Ed., Vol. 1, John Wiley, New York, 1995.
4. Weber, L., Wallbaum, S., Broger, C. and Gubernator, K., *Optimization of the biological activity of combinatorial compound libraries by a genetic algorithm*, *Angew. Chem.*, 107 (1995) 2452–2454; *Angew. Chem. Intern. Ed.*, 34 (1995) 2280–2282.
5. Singh, J., Ator, M.A., Jaeger, E.P., Allen, M.P., Whipple, D.A., Solowej, J.E., Chowdhary, S. and Treasurywala, A.M., *Application of genetic algorithms to combinatorial synthesis: a computational approach to lead identification and lead optimization*, *J. Am. Chem. Soc.*, 118 (1996), 1669–1676.

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6. Morgan, B.P., Holland, D.R., Matthews, B.W. and Barlett, P.A., *Structure-based design of an inhibitor of the zinc peptidase thermolysin*, J. Am. Chem. Soc., 116 (1994) 3251–3260.
7. Kaminski, J.J., Wallmark, B., Briving, C. and Anderson, B.-M., *Antiulcer agents: 5. inhibition of gastric H⁺/K⁺-ATPase by substituted Imidazo[1,2-a]pyridines and related analogs and its implications in modeling the high affinity potassium ion binding site of the gastric proton pump enzyme*, J. Med. Chem., 34 (1991) 533–541.
8. Lauri, G. and Barlett, P.A., *CAVEAT: A program to facilitate the design of organic molecules*, J. Comput.-Aided Mol. Design, 8 (1994) 51–66.
9. Kaplan, A.P. and Barlett, P.A., *Synthesis and evaluation of an inhibitor of carboxypeptidase A with a K_i value in the femtomolar range*, Biochemistry, 30 (1991) 8165–8170.
10. Barlett, P.A. and Marlowe, C.K., *Evaluation of intrinsic binding energy from a hydrogen bonding group in an enzyme inhibitor*, Science, 235 (1987) 569–571.
11. Morgan, B.P., Scholtz, J.M., Ballinger, M.D., Zipkin, I.D. and Barlett, P.A., *Differential binding energy: A detailed evaluation of the influence of hydrogen-bonding and hydrophobic groups on the inhibition of thermolysin by phosphorous-containing inhibitors*, J. Am. Chem. Soc., 113 (1991) 297–307.
12. Merz, K.M. and Kollman, P.A., *Free energy perturbation of the inhibition of thermolysin: Prediction of the free energy of binding of a new inhibitor*, J. Am. Chem. Soc., 111 (1989) 5649–5658.
13. Shuman, R.T., Rothenberger, R.B., Campbell, C.S., Smith, G.F., Gifford-Moore, D.S. and Gesellchen, P.D., *A series of highly selective thrombin inhibitors*, In Smith, J.A. and Rivier, J.E. (Eds) Peptides — chemistry and biology, Proceedings of the 12th American Peptide Symposium, Cambridge, MA, U.S.A., 1991, ESCOM Science Publishers B.V., Leiden, 1992, pp. 801–802.
14. Stanton, J.L., Ksander, G.M., de Jesus, R. and Sperbeck, D.M., *The effect of heteroatom substitution on a series of phosphonate inhibitors of neutral endopeptidase 24.11*, Bioorg. Med. Chem. Lett., 4 (1994) 539–542.
15. Weber, A.E., Steiner, M.G., Krieter, P.A., Colletti, A.E., Tata, J.R., Halgren, T.A., Ball, R.G., Doyle, J.J., Schorn, T.W., Stearns, R.A., Miller, R.R., Siegl, P.K.S., Greenlee, W.J. and Patchett, A.A., *Highly potent, orally active diester macrocyclic human renin inhibitors*, J. Med. Chem. 35 (1992) 3755–3773.
16. Wolfenden, R. and Kati, W.M., *Testing the limits of protein–ligand binding discrimination with transition-state analogue inhibitors*, Acc. Chem. Res., 24 (1991) 209–215.
17. Xiang, S., Short, S.A., Wolfenden, R. and Carter, C.W., *Transition-state selectivity for a single hydroxyl group during catalysis by cytidine deaminase*, Biochemistry, 34 (1995) 4516–4523.
18. Parker, E.M., Griscl, D.A., Iben, L.G. and Shapiro, R.S., *A single amino acid difference accounts for the pharmacological distinctions between the rat and human 5-Hydroxytryptamine_{1B} receptors*, J. Neurochem., 60 (1993) 380–383.
19. Clozel, J.-P. and Fischli, W., *Discovery of remikiren as the first orally active renin inhibitor*, Arzneim.-Forsch. (Drug Research), 43 (1993) 260–262.
20. Li, R.-L., Hansch, C., Matthews, D., Blaney, J.M., Langridge, R., Delcamp, T.J., Susten, S.S. and Freisheim, J.H., *A comparison by QSAR, crystallography, and computer graphics of the inhibition of various dihydrofolate reductases by 5-(X-Benzyl)-2,4-diaminopyrimidines*, Quant. Struct.-Act. Relat., 1 (1982) 1–7.
21. Li, Z., Nguyen, D.T., Kitson, D.H., Bajorath, J., Kraut, J. and Hagler, A.T., *Origin of trimethoprim's pharmacologic activity and differential binding to E. coli and chicken liver dihydrofolate reductases: Long-range electrostatic non-'lock and key' specificity*, Abstract of Presentations, Scientific Seminar Tour 1993, BIOSYM, San Diego, CA, U.S.A., 1993, pp. 14–19.
22. Roques, B.P., Nobel, F., Daugé, V., Fournié-Zaluski, M. and Beaumont, A., *Neutral endopeptidase 24.11: Structure, inhibition and experimental and clinical pharmacology*, Pharmacol. Rev., 45 (1993) 87–146.
23. Roderick, S.L., Fournié-Zaluski, M.C., Roques, B.P. and Matthews, B.W., *Thiorphan and retro-thiorphan display equivalent interactions when bound to crystalline thermolysin*, Biochemistry, 28 (1989) 1493–1497.
24. Slusarchyk, W.A., Robl, J.A., Taunk, P.C., Asaad, M.M., Bird, J.E., DiMarco, J. and Pan, Y., *Dual metalloprotease inhibitors: V. Utilization of bicyclic azepinothiazolidines and azepinonetetrahydrothiazenes in constrained peptidomimetics of mercaptoacyl dipeptides*, Bioorg. Med. Chem. Lett., 7 (1995) 753–758.

25. Hofmann, A., LSD — mein Sorgenkind, dtv/Klett-Cotta, Munich, 1993.
26. Hanson, D.J., *Dioxin toxicity: New studies prompt debate, regulatory action*, Chem. Eng. News, 12 August 1991, 7–14.
27. Mattos, C. and Ringe, D., *Multiple binding modes*, In Kubinyi, H. (Ed.) 3D QSAR in drug design: Theory methods and applications, ESCOM Science Publishers B.V., Leiden, 1993 pp. 226–254.
28. Meyer, E.F., Botos, I., Scapozza, L. and Zhang, D., *Backward binding and other structural surprises*, Persp. Drug Discov. Design, 3 (1993) 168–195.
29. Böhm, H.-J. and Klebe, G., *What can we learn from molecular recognition in protein–ligand complexes for the design of new drugs?*, Angew. Chem., 108 (1996) 2750–2778; Angew. Chem. Intern. Edit., 35 (1996), 2588–2614.
30. Montgomery, J.A. and Niwas, S., *Structure-based drug design*, Chemtech, 23 (1993) 30–37.
31. Montgomery, J.A.; and Secrist III, J.A., *PNP Inhibitors*, Persp. Drug Discov. Design, 2 (1994) 205–220.
32. Kester, W.R., and Matthews, B.W., *Crystallographic study of the binding of dipeptide inhibitors to thermolysin: Implications for the mechanism of catalysis*, Biochemistry, 16 (1977) 2506–2516.
33. Badger, J., Minor, I., Kremer, M.J., Oliveira, M.A., Smith, T.J., Griffith, J.P., Guerin, D.M.A., Krishnaswamy, S., Luo, M., Rossmann, M.G., McKinlay, M.A., Diana, G.D., Dutko, F.J., Fancher, M., Rueckert, R.R. and Heinz, B.A., *Structural analysis of a series of antiviral agents complexed with human rhinovirus 14*, Proc. Natl. Acad. Sci. USA, 85 (1988) 3304–3308.
34. Diana, G.D., Treasurywala, A.M., Bailey, T.R., Oglesby, R.C., Pevear, D.C. and Dutko, F.J., *A model for compounds active against human rhinovirus-14 based on X-ray crystallography data*, J. Med. Chem., 33 (1990) 1306–1311.
35. Bystroff, C., Oatley, S.J. and Kraut, J., *Crystal structures of Escherichia coli dihydrofolate reductase: The NADP⁺ holoenzyme and the folate NADP⁺ ternary complex: Substrate binding and a model for the transition state*, Biochemistry, 29 (1990) 3263–3277.
36. Bolin, J.T., Filman, D.J., Matthews, D.A., Hamlin R.C. and Kraut, J., *Crystal structure of Escherichia coli and Lactobacillus casei dihydrofolate reductase refined at 1.7 Å resolution*, J. Biol. Chem., 257 (1982) 13650–13662.
37. Poulos, T.L. and Howard, A.J., *Crystal structures of metyrapone- and phenylimidazole-inhibited complexes of cytochrome P-450_{cam}*, Biochemistry, 26 (1987) 8165–8174.
38. Mattos, C., Rasmussen, B., Ding, X., Petsko, G.A. and Ringe, D., *Analogous inhibitors of elastase do not always bind analogously*, Nature, Struct. Biol., 1 (1994) 55–58.
39. Masumoto, O., Taga, T., Matsushima, M., Higashi, T. and Machida, K., *Multiple binding of inhibitors in the complex formed by bovine trypsin and fragments of a synthetic inhibitor*, Chem. Pharm. Bull., 38 (1990) 2253–2255.
40. Underwood, D.J., Strader, C.D., Rivero, R., Patchett, A.A., Greenlee, W. and Predergast, K., *Structural model of antagonist and agonist binding to the angiotensin II, AT₁ subtype, G protein coupled receptor*, Chem. Biol., 1 (1994) 211–221.
41. Aquino, C.J., Armour, D.R., Bermann, J.M., Birkemo, L.S., Carr, R.A.E., Croom, D.K., Dezube, M., Dougherty, Jr., R.W., Ervin, G.N., Grizzle, M.K., Head, J.E., Hirst, G.C., James, M.K., Johnson, M.F., Miller, L.J., Queen, K.L., Rimele, T.J., Smith, D.N. and Sugg, E.E., *Discovery of 1,5-Benzodiazepines with peripheral cholecystokinin (CCK-A) receptor agonist activity: 1. Optimization of the Agonist 'Trigger'*, J. Med. Chem., 39 (1996) 562–569.
42. Hirst, G.C., Queen, K.L., Sugg, E.E. and Willson, T.M., *Conversion of acyclic nonpeptide CCK antagonist into CCK agonists*, Bioorg. Med. Chem. Lett., 7 (1997) 511–514.
43. Samanen, J., *GPVIIb/IIIa antagonists*, Ann. Rep. Med. Chem., 31 (1996) 91–100.
44. Engleman, V.W., Kellogg, M.S. and Rogers, T.E., *Cell adhesion integrins as pharmaceutical targets*, Ann. Rep. Med. Chem., 31 (1996) 191–200.
45. Aumailley, M., Gurrath, M., Müller, G., Calvete, J., Timpl, R. and Kessler, H., *Arg-Gly-Asp constrained within cyclic pentapeptides: Strong and selective inhibitors of cell adhesion to vitronectin and laminin fragment PI*, FEBS Lett., 291 (1991) 50–54.
46. Keenan, R., Miller, W., Ali, F., Barton, L., Bondinell, J., Burgess, J., Callahan, J., Calvo, R., Cousins, R., Gowen, M., Huffman, W., Hwang, S., Jakas, D., Ku, T., Kwon, C., Lago, A., Mombouyran, V., Nguyen, T., Ross, S., Samanen, J., Takata, D., Uzinskas, I., Venslavsky, J., Wong, A., Yellin, T. and

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- Yuan, C., *Nonpeptide vitronectin receptor antagonists*, Abstract MEDI 236, 211th ACS National Meeting, 1996.
47. Ariëns, E.J., Wuis, E.W. and Veringa, E.J., *Stereoselectivity of bioactive xenobiotics: A pre-Pasteur attitude in medicinal chemistry, pharmacokinetics and clinical pharmacology*, *Biochem. Pharmacol.*, 37 (1988) 9–18.
 48. Friedman, L. and Miller, J.G., *Odor incongruity and chirality*, *Science*, 172 (1971) 1044–1046.
 49. Höltje, H.-D. and Marrer, S., *A molecular graphics study on structure–action relationships of calcium-antagonistic and agonistic 1,4-dihydropyridines*, *J. Comput.-Aided Mol. Design*, 1 (1987) 23–30.
 50. Kubinyi, H., *QSAR: Hansch analysis and related approaches*, VCH, Weinheim, 1993.
 51. Böhm, H.-J., *The development of a simple empirical scoring function to estimate the binding constant for a protein–ligand complex of known three-dimensional structure*, *J. Comput.-Aided Mol. Design*, 8 (1994) 243–256.
 52. Rum, G. and Herndon, W.C., *Molecular similarity concepts: 5. Analysis of steroid–protein binding constants*, *J. Am. Chem. Soc.*, 113 (1991) 9055–9060.
 53. Good, A.C., Peterson, S.J. and Richards, W.G., *QSAR's from similarity matrices: Technique validation and application in the comparison of different similarity evaluation methods*, *J. Med. Chem.*, 36 (1993), 2929–2937.
 54. Good, A.C., *3D molecular similarity indices and their application in QSAR studies*, In: Dean, P. (Ed.) *Molecular similarity in drug design*, Chapman and Hall, New York, 1995, pp. 24–56.
 55. Martin, Y.C., Lin, C.T., Hetti, C. and DeLazzer, J., *PLS analysis to detect nonlinear relationships between biological potency and molecular properties*, *J. Med. Chem.*, 38 (1995) 3009–3015.
 56. Kubinyi, H., *A General View on Similarity and QSAR Studies*, In *Computer-assisted lead finding and optimization*, Proceedings of the 11th European Symposium on Quantitative Structure–Activity Relationships, Lausanne, Switzerland, 1996; van der Waterbeemd, H., Testa, B. and Folkers, G. (Eds.); Verlag Helvetica Chimica Acta and VCH: Basel, Weinheim, 1997, pp. 7–28.
 57. Klebe, G., Abraham, U. and Mietzner, T., *Molecular similarity indices in a comparative analysis (CoMSIA) of drug molecules to correlate and predict their biological potency*, *J. Med. Chem.*, 37 (1994) 4130–4146.
 58. Kubinyi, H., Hamprecht, F.A. and Mietzner, T., *Three-dimensional quantitative similarity–activity relationships (3D QSiAR), from SEAL similarity matrices*, manuscript submitted for publication.
 59. Kearsley, S.K. and Smith, G.M., *An alternative method for the alignment of molecular structures: Maximizing electrostatic and steric overlap*, *Tetrahedron Comp. Methodol.*, 3 (1990) 615–633.
 60. Klebe, G., Mietzner, T. and Weber, F., *Different approaches toward an automatic alignment of drug molecules: application to sterol mimics, thrombin and thermolysin inhibitors*, *J. Comput.-Aided Mol. Design*, 8 (1994) 751–778.
 61. Cho, S.J. and Tropsha, A., *Cross-validated R^2 guided region selection for comparative molecular field analysis (CoMFA): A simple method to achieve consistent results*, *J. Med. Chem.*, 38 (1995) 1060–1066.