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JOSE LLORED A CAMACHO & CO.

ABOGADOS

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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



No. 10-131479- -00008-0000

Fecha: 2013-01-28 16:33:53 Dep. 2020 DIR.NUEVASCR
Tra. 11 PATENTEIYII Eve: 378 FASENACIONALI
Act. 523 RESPUESTA Folios: 20

Señor

SUPERINTENDENTE DE INDUSTRIA Y COMERCIO

DIVISIÓN DE NUEVAS CREACIONES

E. _____ S. _____ D. _____

Re: PI.-GILEAD SCIENCES, INC.-Solicitud de Patente de Invención en Fase
Nacional PCT para "NUCLEÓSIDOS DE IMIDAZOL[1,5-
F][1,2,4]TRIAZINIL, IMIDAZOL[1,2-F][1,2,4]TRIAZINIL Y
[1,2,4]TRIAZOLO[4,3-F][1,2,4] TRIAZINIL PARA TRATAMIENTO
ANTIVIRAL".-Clase C07D 487/04.-Expediente número 10-131.479.-

1. Me refiero al auto dictado por ese Despacho y notificado por fijación en lista de 30 de Octubre de 2012.

2. Con el fin de responder las objeciones expuestas por el señor Examinador en su Concepto Técnico correspondiente al Oficio **No. 19681**, con respecto a la presencia dentro del capítulo reivindicatorio de materia que estaría exenta de patentabilidad y relacionada con usos o segundos usos, y con respecto al Nivel Inventivo de la presente solicitud, me permito dirigir su atención a las siguientes modificaciones y argumentos técnicos por medio de los cuales se superan completamente dichas objeciones.

3. **MODIFICACIONES EN EL CAPÍTULO REIVINDICATORIO**

Tomando en consideración los diferentes señalamientos realizados por el señor Examinador en su Oficio antes referenciado, me permito manifestar que es deseo del solicitante modificar el capítulo reivindicatorio actualmente en estudio con el propósito de definir de manera más adecuada el objeto de la presente invención e identificar con mayor claridad y concisión sus características técnicas novedosas e inventivas.

Respecto a estas modificaciones, me permito resaltar que no implican de ninguna manera una ampliación al alcance de la solicitud, tal como fue originalmente presentada, toda vez que se está limitando el alcance de la solicitud de conformidad con las realizaciones preferidas de la invención, como se presenta a continuación:

- (i) **Reivindicaciones 1 a 7:** Corresponden respectivamente a las reivindicaciones 1 a 7 actualmente presentadas, las cuales no han sido modificadas.
- (ii) **Reivindicación 8:** Corresponde a la reivindicación 9 actualmente presentada, en donde únicamente se han ajustado sus dependencias de conformidad con la nueva numeración ahora dada al capítulo reivindicatorio.
- (iii) **Reivindicación 9:** Se refiere a la materia contenida en las reivindicaciones 11 y 12 actualmente presentadas.
- (iv) **Reivindicación 10:** Corresponde a la reivindicación 16 actualmente presentada, la cual no ha sido modificada.
- (v) **Reivindicaciones 11 a 13:** Corresponden de manera respectiva a las reivindicaciones 18 a 20 actualmente presentadas, en las que se han ajustado sus dependencias de conformidad con la nueva numeración dada al capítulo reivindicatorio.
- (vi) Por último, las reivindicaciones 8, 10, 13 a 15, 17, 21 a 28 actualmente presentadas han sido **eliminadas** del capítulo reivindicatorio ahora modificado.

En vista de lo anterior, el capítulo reivindicatorio modificado que se adjunta a este memorial queda constituido por **13 reivindicaciones** que reclaman el objeto de la presente solicitud de manera clara y concisa a través de las características técnicas esenciales de sus realizaciones más preferidas.

4. CAPÍTULO REIVINDICATORIO: PRESENCIA DE MATERIA EXENTA DE PATENTABILIDAD Y RELACIONADA CON USOS Y/O SEGUNDOS USOS

A este respecto el señor Examinador manifiesta que las reivindicaciones 21 a 26 corresponderían a materia exenta de patentabilidad.

Adicionalmente, señala que la reivindicación 27 correspondería a un uso lo cual no es patentable.

Acatando estos señalamientos me permito manifestar que las reivindicaciones 21 a 27, entre otras, del capítulo reivindicatorio actualmente presentado han sido eliminadas del capítulo reivindicatorio ahora modificado, con lo cual se superan por completo las objeciones que fueron presentadas por el señor Examinador.

Así las cosas, el capítulo reivindicatorio ahora modificado es totalmente claro, conciso, se encuentra debidamente sustentado en el texto de la descripción, y se refiere solamente a materia patentable ajustándose así totalmente a las disposiciones de la Decisión Andina 486 de 2000.

5. NIVEL INVENTIVO DE LA SOLICITUD

Finalmente, el señor Examinador objeta el nivel inventivo de las reivindicaciones 1 a 18 de la presente invención con base en las enseñanzas de los documentos Dunfield P. J. et. al., "Synthesis of C-ribosyl imidazo[2,1-f][1,2,4]triazines as inhibitors of adenosine and AMP deaminases", **J. Chem. Soc., Perkin Trans. 1, 1999, pp. 2929-2936 (D1) y US 20040067901 (D2).**

Con relación al documento D1 el señor Examinador manifiesta que es el documento del estado del arte más cercano a la invención el cual se diferencia de esta última en que para la invención definida en la reivindicación 1, en la posición 5 del anillo [2,1-f][1,2,4] triazina existe un grupo de sustituyentes que incluyen (halógeno, amino, ciano, entre otros) y el documento D1 menciona un sustituyente hidrógeno para dicha posición.

Por otro lado, ese Despacho sostiene que la diferencia entre la invención definida en la reivindicación 1 y el compuesto I del documento D2 corresponde a que la invención define como sustituyente en la posición 2 de anillo tetrahidrofurano una base nitrogenada seleccionada entre imidazo [2,1-f][1,2,4]triazina y [1,2,4]triazolo [3,4-f][1,2,4] triazina y el documento D2 tiene como sustituyente un grupo de base nitrogenada que incluyen 9-H-purina, 3H-pirazolo[4,3-d] pirimidina, 1H-pirazolo[3,4-d] pirimidina y 7H-pirrolo [3,2-d] pirimidina.

Así las cosas, esa Oficina manifiesta que el problema técnico objeto que pretende resolver esta invención se puede formular como "*cómo modificar los sustituyentes del compuesto 3 del documento D1 para sí obtener nuevos compuesto alternativos útiles para el tratamiento de infecciones por virus de la familia Flaviviridae.*"

A lo anterior añade que "*Sin embargo, dado que el documento D1 refiere que el 3-β-D-ribofuranósido de una nueva imidazo [2,1-f][1,2,4] triazina (Compuesto 3) es isomérico e isoelectrónico con el nucleósido deaminoformicina (compuesto 1), por lo cual una persona versada en la materia entendería que el anillo imidazo[2,1-f][1,2,4] triazina es isomérico e isoelectrónico al anillo 3H-pirazolo[4,3-d]pirimidina para los ribufuranósidos y que estos poseen actividad antiviral y que el documento D2 refiere que el anillo 3H-pirazolo [4,3-d] pirimidina puede ser sustituyente de una*

serie de compuestos análogos a nucleósidos inhibidores de la RNA-polimerasa viral dependiente de RNA en especial inhibidores de la replicación del virus de la hepatitis C (HCV) perteneciente a la familia Flaviviridae."

Así las cosas, el señor Examinador concluye que *"una persona del oficio normalmente versada en la materia, estaría motivada o tendría suficientemente expectativa de éxito modificar los sustituyentes del compuesto 3 de D1 para así obtener nuevos compuestos alternativos útiles para el tratamiento de infecciones por virus de la familia Flaviviridae de la manera que está sugerida en el documento D2. Por lo cual la reivindicación 1 se considera obvia."* (Sic)

Además manifiesta que las reivindicaciones dependientes 2 a 18 no mencionan características inventivas adicionales y por lo tanto no se pueden considerar tampoco inventivas.

Visto lo anterior y con el ánimo de demostrar que el nuevo capítulo reivindicatorio de la solicitud en cuestión presenta un inequívoco carácter inventivo frente a la combinación de las enseñanzas de los documentos D1 y D2, atentamente me permito presentar los siguientes argumentos y consideraciones técnicas:

En primer lugar me permito manifestar que el señor Examinador considera que el documento D1 es el documento del estado del arte más cercano a la presente invención debido a los compuestos 1 y 3 allí revelados. Sin embargo, es necesario dirigir la atención de ese Despacho al hecho que **el documento D1 falla en enseñar la sustitución en la posición 5 del anillo de [2,1-f][1,2,4] triazina que tienen los compuestos reclamados por la presente invención.**

Adicionalmente, me permito resaltar que a partir de la lectura del documento D1 salta a la vista que **dicha referencia enseña el uso de los compuestos identificados como inhibidores de adenosina y AMP desaminasas pero falla en enseñar o sugerir que los compuestos allí divulgados puedan ser útiles para tratamiento antiviral, como lo son los compuestos revelados por la presente solicitud.**

Así las cosas, a la luz de las enseñanzas de D1 salta a la vista que una persona medianamente versada en la materia no podría tener una expectativa o sugerencia de que un compuesto para utilizarse como inhibidor de adenosina y AMP desaminasas podría ser efectivo para el tratamiento de infecciones virales causadas por *Flaviviridae*.

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Sumado a lo anterior, me permito manifestar que D1 cita el artículo de Glazer¹ (que se adjunta a este escrito) para referirse a que los inhibidores de la enzima adenosina desaminasa son de interés en "*Quimioterapia viral*", como se señala en las líneas 5 y 6 de la columna izquierda de la primera página de D1. De manera más particular, me permito señalar que la única mención que hace el documento de Glazer a una inhibición antiviral específica concierne a la inhibición de "*herpes simplex I y II*", así como también de *virus de RNA que producen estomatitis y sarampión*" (véase los primeros dos párrafos de la columna izquierda de la página 231).

Consecuentemente, queda una vez más expuesto el hecho que el documento D1 no enseña ni sugiere la actividad antiviral contra *Flaviviridae* de los compuestos revelados en tal referencia del estado del arte.

Por otro lado, me permito señalar que el documento enseña derivados de nucleósidos para su uso como inhibidores de la RNA polimerasa viral dependiente de RNA. Sin embargo, de la lectura de D2 es claro que tal referencia falla en enseñar compuestos con el núcleo central que tienen los compuestos objeto de la presente invención.

Por lo tanto, **a partir de las enseñanzas de los documentos D1 y D2 la persona medianamente versada en la materia no se vería motivada a sustituir específicamente la posición 5 del anillo de [2,1-f][1,2,4] triazina del compuesto 3 de D1 con los sustituyentes enseñados en D2 y esto básicamente porque a partir de las enseñanzas de los documentos D1 y D2 dicha persona medianamente versada en la materia no tendría una motivación para ello y mucho menos una expectativa razonable de éxito de que los compuestos así obtenidos fueran efectivos para el tratamiento de infecciones virales causadas por *Flaviviridae*.**

Así las cosas, me permito muy respetuosamente llamar la atención a ese Despacho al hecho que el análisis presentado en el referenciado Concepto Técnico estaría enmarcado dentro de lo que en el ámbito de las patentes se conoce como análisis "hindsight", o análisis "Ex post facto", toda vez que conociendo de antemano la invención el señor Examinador está seleccionando partes específicas de las enseñanzas contenidas dentro de los documentos D1 y D2 y las agrupa de manera particular a fin de construir la materia reclamada por la presente solicitud sin una motivación en el estado del arte de para qué hacer tales agrupaciones, lo cual de ninguna manera podría ser evidente para la persona versada en la materia y mucho menos en el momento en el cual se desarrolló la invención.

¹ Glazer R. I., "Adenosine Deaminase Inhibitors: Their Role in Chemotherapy and Immunosuppression", Cancer Chemother. Pharmacol., 1980, 4, pp. 227-235.

En este sentido vale la pena resaltar que al tener conocimiento de la invención es de esperarse que parezcan obvias dichas selecciones particulares de los sustituyentes de D2 y su inclusión específicamente en la posición 5 del anillo de [2,1-f][1,2,4] triazina del compuesto 3 de D1, toda vez que ya se sabe de antemano que efectivamente tales cambios resuelven un problema técnico (el cual vale la pena resaltar no se identifica ni se sugiere por ninguno de D1 o D2 o su combinación); sin embargo, esta información no era conocida por el inventor en el momento de desarrollar la invención, por lo que no podría tener certeza de la utilidad de realizar esta selección particular de sustituyentes y su presencia sobre una posición particular, posición 5, del anillo de [2,1-f][1,2,4] triazina de los compuestos de la presente invención, y esto dejando a un lado los sustituyentes particulares del otro anillo de la molécula análogo de nucleósido.

Teniendo en cuenta los anteriores argumentos, me permito señalar que dentro del ámbito de las patentes es bien conocido que para la evaluación de nivel inventivo de una solicitud de patente no se evalúa el hecho de si la persona medianamente versada en la materia podría intentar una o más alternativas y llegar a la materia de la solicitud en estudio por adaptación o modificación de las enseñanzas de los documentos del estado del arte, sino si dicha persona en realidad hubiera tenido una motivación a partir de las enseñanzas de tales documentos para hacer las adaptaciones o modificaciones en los compuestos allí revelados para obtener otros (los de la presente solicitud) **con la expectativa de resolver el problema técnico objetivo o la expectativa de alguna mejora o ventaja técnica.**

Tomando en consideración los argumentos anteriores, salta a la vista que **los compuestos de formula I reclamados por la reivindicación 1 del capítulo reivindicatorio modificado de la presente solicitud son inventivos frente a las enseñanzas de los documentos D1 y D2 toda vez que a partir de estos documentos la persona medianamente versada en la materia no encontraría ninguna motivación que lo condujera de manera obvia ni evidente a realizar la selección particular de grupos sustituidos en 2', 3' o 4' junto con un heteroanillo nitrogenado central que se une por uno de sus carbonos y que este anillo central a su vez tenga determinados sustituyentes sobre su posición 5 (de manera que correspondan a los compuestos de la presente invención los cuales son efectivos para el tratamiento de infecciones virales causadas por *Flaviviridae*) pues a partir de los documentos D1 y D2 la persona versada en la materia no podría tener una exceptiva de la utilidad de tales cambios.**

Dentro de este marco de ideas me permito referirme al señalamiento que hace el Tribunal de Justicia de la Comunidad Andina con respecto a las definiciones de "obvio" y "evidente" en la evaluación del Nivel Inventivo de una solicitud de patente²:

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

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

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

Así, **dado que a partir de los documentos D1 y D2 no "salta a la vista de manera clara y directa" compuestos con cada una de las características estructurales de los compuestos reclamados por la reivindicación 1 del capítulo reivindicatorio modificado de la presente solicitud; y que de las enseñanzas de dichas referencias no se tiene ninguna "certeza clara y manifiesta" de que tales selecciones de sustituyentes y sus posiciones particulares sobre los anillos de los compuestos de D1 y D2 darían lugar a otro compuestos, los de la presente invención, que serían efectivos para el tratamiento de infecciones virales causadas por *Flaviviridae*, queda de manifiesto que la materia reclamada por la reivindicación 1 del capítulo reivindicatorio modificado de la presente solicitud conlleva nivel inventivo frente a la combinación de las enseñanzas del estado del arte comprendido en D1 y D2, cumpliendo de esta manera la materia reclamada por la presente solicitud con las disposiciones de patentabilidad establecidas en el Artículo 18 de la Decisión 486 de 2000.**

² Tribunal de Justicia de la Comunidad Andina, PROCESO 192-IP-2005

De la misma manera, las reivindicaciones 2 a 13 del capítulo reivindicatorio ahora modificado también gozan de altura inventiva frente a las enseñanzas de los documentos D1 y D2 toda vez que son reivindicaciones dependientes de la reivindicación 1 ahora modificada cuya materia es inventiva frente a tales documentos del estado del arte.

6. CONCESION DE LA PATENTE EN OTROS PAISES

Finalmente y aparte de lo anteriormente mencionado, me permito señalar que la solicitud de patente en cuestión ya ha sido aceptada por otras Oficinas de patentes como por ejemplo la Oficina de Propiedad Industrial de la Unión Europea la cual ha concedido la patente **EP 2280973 B1**.

Ahora bien, ante todo es preciso señalar que con la información mencionada anteriormente de ninguna manera pretendo desconocer la independencia de la que goza la Superintendencia de Industria y Comercio al momento de analizar los méritos de patentabilidad de una solicitud.

Sin embargo, sí considero de gran importancia resaltar el hecho de que la materia técnica revelada en la solicitud de patente en cuestión ha sido catalogada como novedosa, inventiva e industrialmente aplicable por los Examinadores de otra Oficina de Propiedad Intelectual, hecho que evidentemente representa un claro indicio de que la materia técnica revelada en la solicitud de patente en estudio cumple a cabalidad con los requisitos de patentabilidad establecidos en la Decisión 486 de 2000.

7. Anexo al presente memorial me permito presentar:

- (i) Recibo Número 13-001574, expedido por la Superintendencia de Industria y Comercio que acredita el pago de las reivindicaciones adicionales.
- (ii) Copia de las páginas 143 a 151 del capítulo reivindicatorio modificado, el cual consta de 13 reivindicaciones.
- (iii) Copia del document de Glazer R. I., "*Adenosine Deaminase Inhibitors: Their Role in Chemotherapy and Immunosuppression*", Cancer Chemother. Pharmacol., 1980, 4, pp. 227-235.

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

Señor Superintendente,



ALICIA LLORED RICAURTE

T. P. 53.215

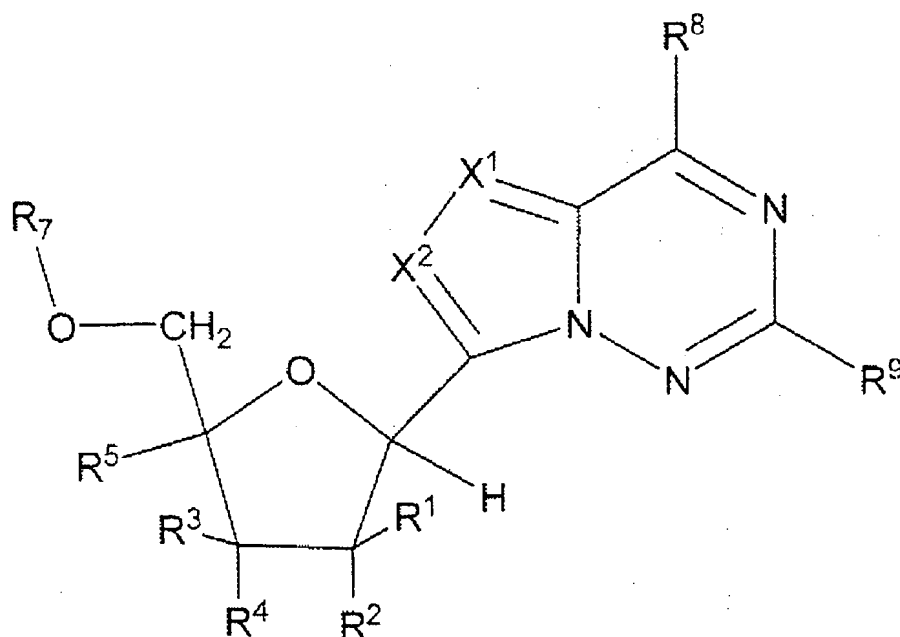
Dirección para Notificaciones: **notificacionespatentes@lloredacamacho.com**

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 Industria y Comercio SUPERINTENDENCIA	RECIBO DE CAJA			No. 13 - 001574	
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*** Conceptos Pagados ***					
CANT.	RENTISTICO	CONCEPTO	Vr.UNDITARIO	Vr.CONCEPTO	
3 50005-01-01	SOLICITUDES	1607 REIVINDICACION PATENTE UNITARIA ADICIONAL A LAS 10 INIC	30.000.00	90.000.00	
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SON: **NOVENTA MIL PESOS MONEDA CORRIENTE***					
Responsable: 					

Recibo de Caja Aplicado al Expediente No. _____					
Sede Centro: Carrera 13 No. 27 - 00 Pisos 3,4,5 y 10 Bogotá, D.C.- Colombia					
Web: www.sic.gov.co e-mai: info@sic.gov.co Conmutador: (571) 5870000 Fax: (571) 5870284 Línea: 018000-910165 Call Center: (571) 6513240					
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REIVINDICACIONES

1. Un compuesto de Fórmula I:



Formula I

o una sal farmacéuticamente aceptable del mismo;

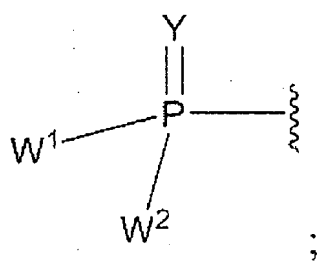
- 5 en donde:

al menos uno de R^1 , R^2 , R^3 , R^4 , o R^5 es $N(R^a)_2$, N_3 , CN , NO_2 , $S(O)_nR^a$, halógeno, alquilo(C_1-C_8), carbociclilalquilo(C_4-C_8), alquilo(C_1-C_8) sustituido, alquenilo(C_2-C_8), alquenilo(C_2-C_8) sustituido, alquinilo(C_2-C_8), alquinilo (C_2-C_8)sustituido o aril-alquilo(C_1-C_8) y cada R^1 , R^2 , R^3 , R^4 , o R^5 restante es,
 10 independientemente, H , OR^a , $N(R^a)_2$, N_3 , CN , NO_2 , $S(O)_nR^a$, halógeno, alquilo(C_1-C_8), carbocicloalquilo(C_4-C_8), alquilo(C_1-C_8) sustituido, alquenilo(C_2-C_8), alquenilo(C_2-C_8)sustituido, alquinilo(C_2-C_8), alquinilo(C_2-C_8) sustituido o aril-alquilo(C_1-C_8) o cualquier dos R^1 , R^2 , R^3 , R^4 , o R^5 sobre átomos de carbono adyacentes al ser tomados juntos son $-O(CO)O-$ o al ser tomados juntos con los átomos de carbono del
 15 anillo a los cuales están unidos forman un doble enlace;

cada n es independientemente 0, 1, ó 2;

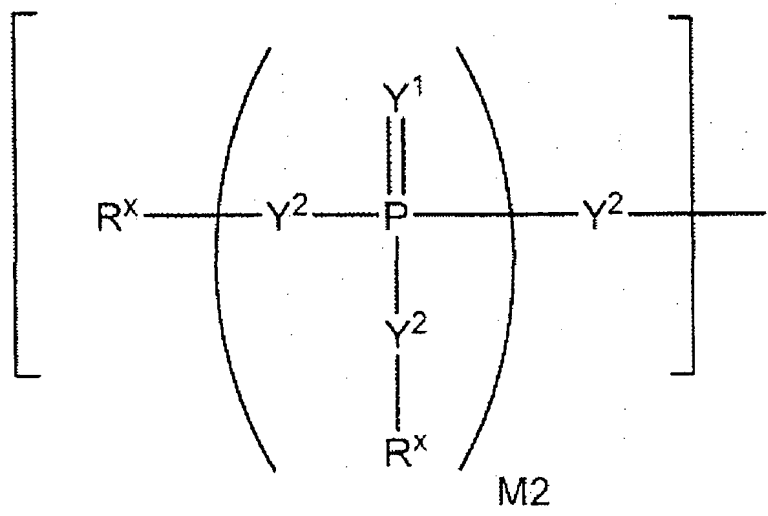
cada R^a es independientemente H , alquilo(C_1-C_8), alquenilo(C_2-C_8), alquinilo(C_2-C_8), aril-alquilo(C_1-C_8), carbociclilalquilo(C_4-C_8), $-C(=O)R^{11}$, $-C(=O)OR^{11}$, $-C(=O)NR^{11}R^{12}$, $-C(=O)SR^{11}$, $-S(O)R^{11}$, $-S(O)_2R^{11}$, $-S(O)(OR^{11})$, $-S(O)_2(OR^{11})$, -
 20 $SO_2NR^{11}R^{12}$;

R^7 es H, $-C(=O)R^{11}$, $-C(=O)OR^{11}$, $-C(=O)NR^{11}R^{12}$, $-C(=O)SR^{11}$, $-S(O)R^{11}$, $-S(O)_2R^{11}$, $-S(O)(OR^{11})$, $-S(O)_2(OR^{11})$, $-SO_2NR^{11}R^{12}$, o



5 cada Y o Y^1 es, independientemente, O, S, NR, $^+N(O)(R)$, N(OR), $^+N(O)(OR)$, o N-NR₂;

W^1 y W^2 , al ser tomados juntos, son $-Y^3(C(R^y)_2)_3Y^3-$; o uno de W^1 o W^2 junto con ya sea R^3 o R^4 es $-Y^3-$ y el otro de W^1 o W^2 es la Fórmula Ia; o W^1 y W^2 son cada uno, independientemente, un grupo de la Fórmula Ia:



Formula Ia

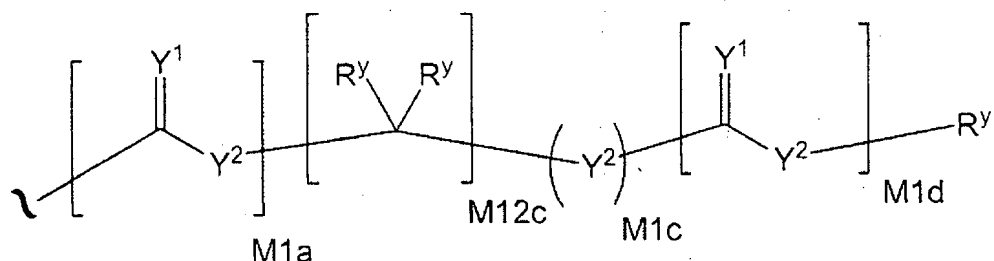
10 en donde:

cada Y^2 es independientemente un enlace, O, CR₂, NR, $^+N(O)(R)$, N(OR), $^+N(O)(OR)$, N-NR₂, S, S-S, S(O), o S(O)₂;

cada Y^3 es independientemente O, S, o NR;

M2 es 0, 1 ó 2;

15 cada R^x es independientemente R^y o la fórmula:



en donde:

cada M1a, M1c, y M1d es independientemente 0 ó 1 ;

M12c es 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 ó 12;

- 5 cada R^y es independientemente H, F, Cl, Br, I, OH, R, $-C(=Y^1)R$, $-C(=Y^1)OR$, $-C(=Y^1)N(R)_2$, $-N(R)_2$, $-N^+(R)_3$, $-SR$, $-S(O)R$, $-S(O)_2R$, $-S(O)(OR)$, $-S(O)_2(OR)$, $-OC(=Y^1)R$, $-OC(=Y^1)OR$, $-OC(=Y^1)(N(R)_2)$, $-SC(=Y^1)R$, $-SC(=Y^1)OR$, $-SC(=Y^1)(N(R)_2)$, $-N(R)C(=Y^1)R$, $-N(R)C(=Y^1)OR$, $-N(R)C(=Y^1)N(R)_2$, $-SO_2NR_2$, $-CN$, $-N_3$, $-NO_2$, $-OR$, o W^3 ; o al ser tomados juntos, dos R^y en el mismo átomo de carbono forman un anillo carbocíclico de 3 a 7 átomos de carbono;
- 10

cada R es independientemente H, alquilo(C_1-C_8), alquilo(C_1-C_8) sustituido, alquenilo(C_2-C_8), alquenilo(C_2-C_8) sustituido, alquinilo(C_2-C_8), alquinilo(C_2-C_8) sustituido, arilo C_6-C_{20} , arilo C_6-C_{20} sustituido, heterociclilo C_2-C_{20} , heterociclilo C_2-C_{20} sustituido, arilalquilo o arilalquilo sustituido;

- 15 W^3 es W^4 o W^5 ; W^4 es R, $-C(Y^1)R^y$, $-C(Y^1)W^5$, $-SO_2R^y$, o $-SO_2W^5$; y W^5 es un carbociclo o un heterociclo en donde W^5 está independientemente sustituido con 0 a 3 grupos R^y ;

cada X^1 o X^2 es independientemente $C-R^{10}$ o N en donde por lo menos uno de X^1 o X^2 es N;

- 20 cada R^8 es, independientemente, halógeno, $NR^{11}R^{12}$, $N(R^{11})OR^{11}$, $NR^{11}NR^{11}R^{12}$, N_3 , NO, NO_2 , CHO, CN, $-CH(=NR^{11})$, $-CH=NHNR^{11}$, $-CH=N(OR^{11})$, $-CH(OR^{11})_2$, $-C(=O)NR^{11}R^{12}$, $-C(=S)NR^{11}R^{12}$, $-C(=O)OR^{11}$, alquilo(C_1-C_8), alquenilo(C_2-C_8), alquinilo(C_2-C_8), carbociclilalquilo(C_4-C_8), arilo opcionalmente sustituido, heteroarilo opcionalmente sustituido, alquilo- $C(=O)(C_1-C_8)$, $-S(O)_n$ -alquilo(C_1-C_8) o aril-alquilo(C_1-C_8), OR^{11} o SR^{11} ;
- 25

cada R^9 o R^{10} es independientemente H, halógeno, $NR^{11}R^{12}$, $N(R^{11})OR^{11}$, $NR^{11}NR^{11}R^{12}$, N_3 , NO, NO_2 , CHO, CN, $-CH(=NR^{11})$, $-CH=NHNR^{11}$, $-CH=N(OR^{11})$, $-CH(OR^{11})_2$, $-C(=O)NR^{11}R^{12}$, $-C(=S)NR^{11}R^{12}$, $-C(=O)OR^{11}$, R^{11} , OR^{11} o SR^{11} ;

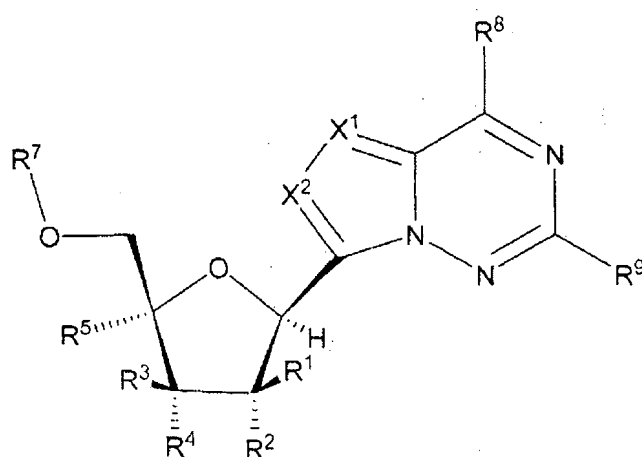
cada R^{11} o R^{12} es independientemente H, alquilo (C_1-C_8), alqueno(C_2-C_8), alquino(C_2-C_8), carbociclilalquilo(C_4-C_8), arilo opcionalmente sustituido, heteroarilo opcionalmente sustituido, $-C(=O)$ -alquilo(C_1-C_8), $-S(O)_n$ -alquilo(C_1-C_8) o aril-alquilo(C_1-C_8); o R^{11} y R^{12} tomados junto con un átomo de nitrógeno al que ambos están unidos forman un anillo heterocíclico de 3 a 7 miembros en donde cualquier átomo de carbono de dicho anillo heterocíclico puede ser reemplazado
10 opcionalmente con $-O-$, $-S-$ o $-NR^3-$;

en donde cada alquilo(C_1-C_8), alqueno(C_2-C_8), alquino(C_2-C_8) o aril-alquilo(C_1-C_8) de cada R^1 , R^2 , R^3 , R^4 , R^5 , R^{11} o R^{12} está, independientemente, opcionalmente sustituido con uno o más halo, hidroxilo, CN, N_3 , $N(R^8)_2$ o OR^a ; y en donde uno o más de los átomos de carbono no terminales de cada uno de dichos alquilo(C_1-C_8) puede ser opcionalmente reemplazado con $-O-$, $-S-$ o $-NR^a-$.
15

2. Un compuesto de acuerdo con la reivindicación 1 en donde R^8 es halógeno, $NR^{11}R^{12}$, $N(R^{11})OR^{11}$, $NR^{11}NR^{11}R^{12}$, OR^{11} o SR^{11} .

3. Un compuesto de acuerdo con las reivindicaciones 1 ó 2 en donde R^9 es H o $NR^{11}R^{12}$.

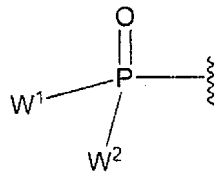
20 4. Un compuesto de acuerdo con cualquiera de las reivindicaciones 1-3 representado por la Fórmula II



Formula II

en donde cada Y y Y^1 es O.

5. Un compuesto de acuerdo con cualquiera de las reivindicaciones 1-4 en donde R^7 es H o



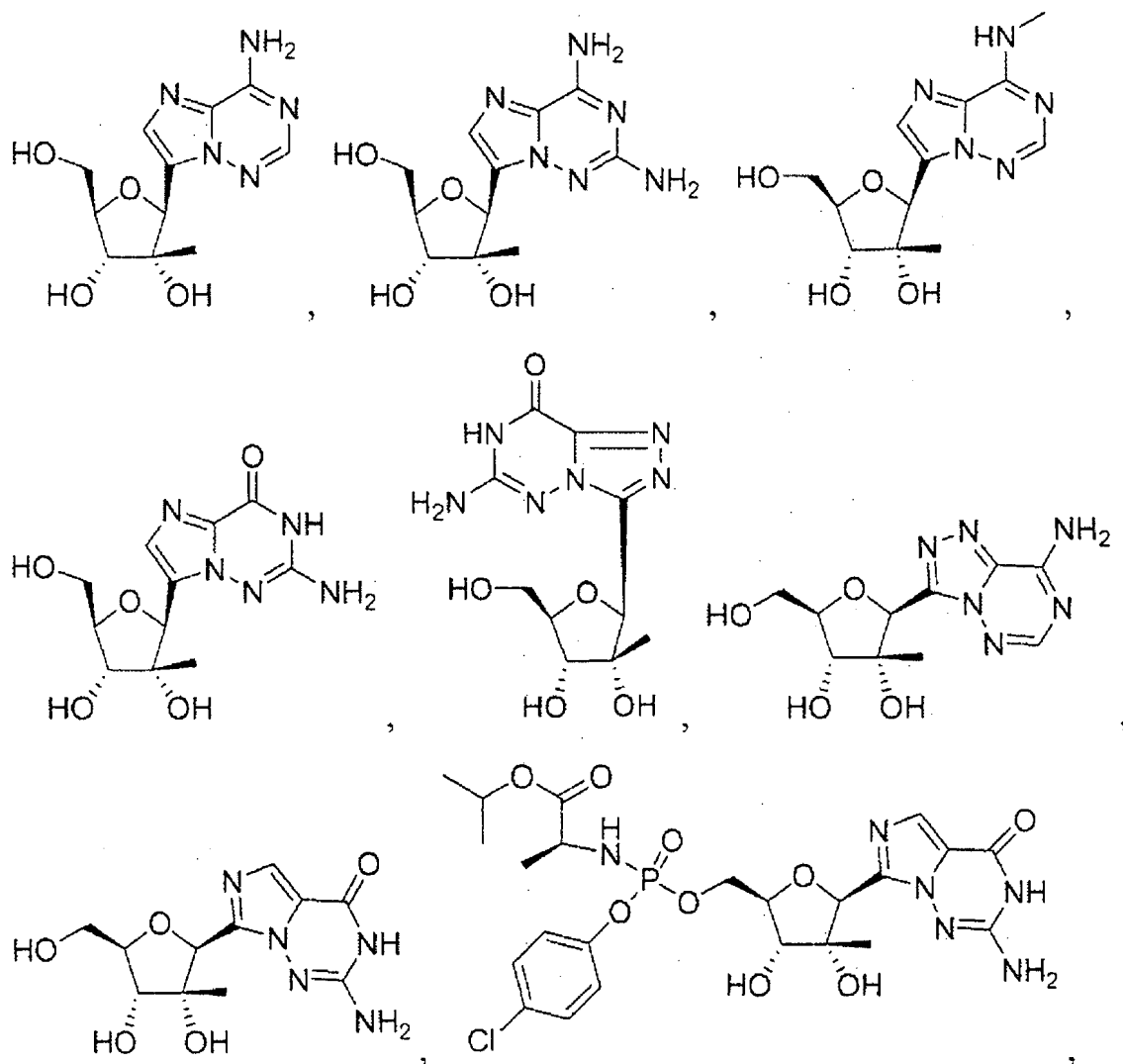
6. Un compuesto de acuerdo con cualquiera de las reivindicaciones 1-5 en donde X^1 es N y R^3 es H.

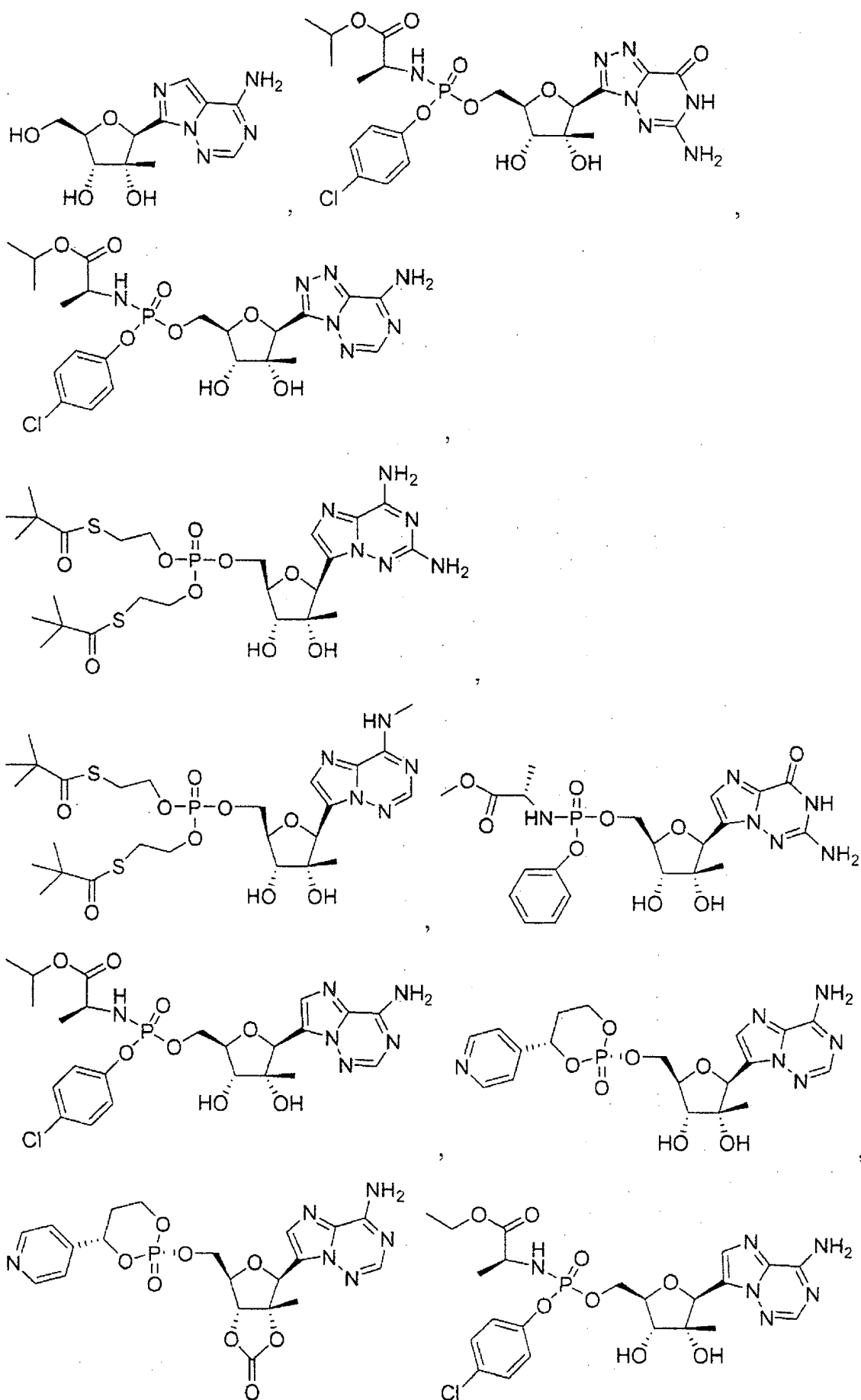
7. Un compuesto de acuerdo con cualquiera de las reivindicaciones 1-6 en donde cada R^2 y R^4 es OR^a .

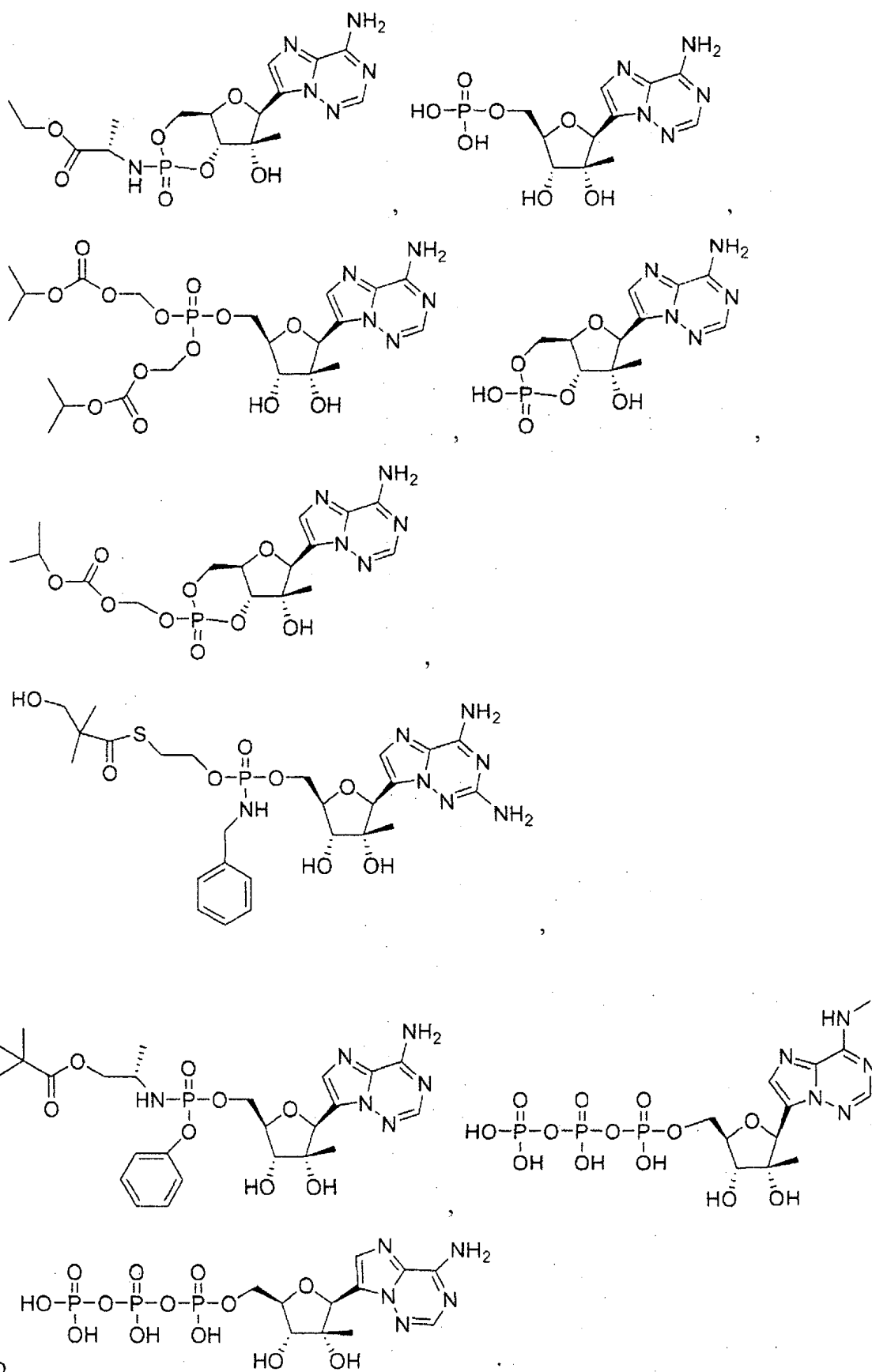
8. Un compuesto de acuerdo con cualquiera de las reivindicaciones 1-7 en donde X^2 es CH y R^1 se selecciona de metilo, CH_2OH , CH_2F , etenilo, o etinilo.

9. Un compuesto de acuerdo con cualquiera de las reivindicaciones 1-8 en donde R^1 es metilo y R^5 es H.

10. Un compuesto de acuerdo con la reivindicación 1 que es







o una sal farmacéuticamente aceptable del mismo.

11. Una composición farmacéutica que comprende una cantidad terapéuticamente efectiva de un compuesto de cualquiera de las reivindicaciones 1 a 10 y un portador farmacéuticamente aceptable.

12. La composición farmacéutica de la reivindicación 11 que comprende al menos un agente terapéutico adicional.

13. La composición farmacéutica de la reivindicación 12, en donde dicho agente terapéutico adicional se selecciona del grupo que consiste de interferones, análogos
5 de ribavirina, inhibidores de la proteasa NS3, inhibidores NS5a, inhibidores de la polimerasa NS5b, inhibidores de la alfa-glucosidasa 1, inhibidores de ciclofilina, hepatoprotectores, inhibidores no nucleósidos del VHC y otros fármacos para tratar el VHC.

Adenosine Deaminase Inhibitors: Their Role in Chemotherapy and Immunosuppression

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Introduction

The search for inhibitors of adenosine deaminase (ADA) is an area of drug development that has been actively pursued for the past 15 years. The development of the antibiotics coformycin and dCF, which are stoichiometric tight-binding inhibitors, has sparked new interest in this area. These drugs have greatly improved the therapeutic activity, and in some instances also the therapeutic index, of anticancer agents which are adenosine analogs. dCF also possesses lymphocytopenic and immunosuppressive activities. Because of its unusual combination of therapeutic activities, there is presently intense clinical interest in dCF both as a single agent and in combination with antitumor and antiviral adenosine analogs. On the other hand, aliphatic alcohol analogs of adenine, such as EHNA, possess intrinsic antiviral activity as well as potentiating the anticancer activity of adenosine analogs.

The recent proliferation of reports concerned with the use of ADA inhibitors in combination with antitumor agents, as well as with adenosine and 2'-deoxyadenosine, has prompted a need for the collation and comparative assessment of these data. In this review I have concentrated upon the pharmacological properties of ADA inhibitors, their use in studying the mechanisms of cytotoxicity of adenosine analogs and metabolites, and the preliminary pharmacokinetic and clinical findings of these drugs.

Structure-Activity and Kinetic Characteristics

Initially, synthetic analogs of adenosine were found to be weak competitive inhibitors of ADA with an ID_{50} in the range of 10^{-5} to 10^{-4} M [22, 27, 70, 93]. The structure-activity requirements optimal for activity were 6-amino and 9-ribosyl or 9-(2-hydroxyalkyl) functionalities. It was not until the rational design and synthesis of EHNA (Fig. 1) with an ID_{50} of 10^{-8} M that a relatively tight-binding competitive inhibitor became available [69].

The most potent inhibitors of ADA have evolved from the isolation of natural products. The purification of the antibiotics coformycin and dCF (Fig. 1) by Umezawa's laboratory [56, 68] and by Parke-Davis investigators [92], respectively, have provided prototypes of unique tight-binding transition state inhibitors with an ID_{50} of 10^{-8} to 10^{-9} M for coformycin [6, 14] and 10^{-9} to 10^{-11} M for dCF [4, 7, 21, 40], depending on the molecular species of ADA and the amount of enzyme present in the assay.

The tetrahedral configuration of the 8-hydroxy of the diazepine ring moiety of the coformycins resembles the transition state intermediate in the hydrolytic deamination of adenosine (Fig. 2). This unique structure enables them to function as stoichiometric transition state inhibitors, with the ribose and 2'-deoxyribose moieties providing added stabilization of the ADA-coformycin complex [91].

The first clue that the coformycin-type of ADA inhibitors were of the tight-binding class was provided by Sawa et al. [68]. The rate of inhibition by coformycin was found to increase with the length of preincubation to produce a mixed type of inhibition. These effects were confirmed and extended in elegant kinetic studies [14, 15], where it was shown that the extent and rate of inhibition was a dual function of enzyme concentration and length of incubation. The calculated K_i for coformycin with erythrocytic ADA

Abbreviations: ADA, adenosine deaminase; dCF, 2'-deoxycoformycin; EHNA, erythro-9-(2-hydroxy-3-nonyl)adenine; ID_{50} , dose that inhibits 50%; SAH, S-adenosyl-homocysteine
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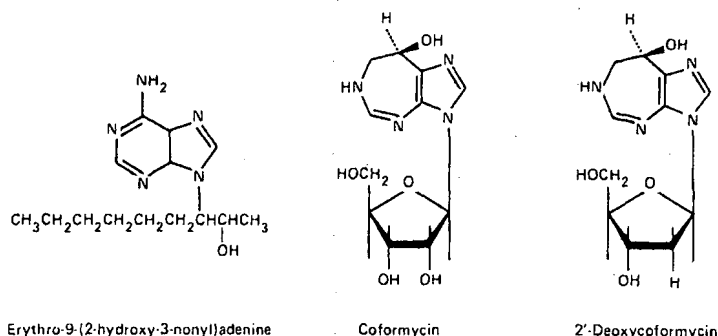


Fig 1. Structures of EHNA, coformycin and 2'-deoxycosphycin

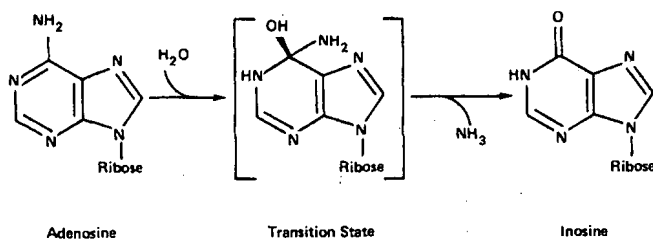


Fig. 2. Deamination of adenosine by adenosine deaminase, showing the transition state intermediate

was $2.2 \times 10^{-10} M$. Similar kinetic features were evident in subsequent studies with dCF where the K_i was calculated from the association and dissociation rate constants to be $2.5 \times 10^{-12} M$ [4]. In contrast, the K_i for EHNA was $1.6 \times 10^{-9} M$ [4]. The 100- and 1,000-fold lower K_i for coformycin and dCF, respectively, vs EHNA is a direct quantitative result of their correspondingly lower dissociation rate constants. A factor that must also be considered is the recently discovered differential sensitivity of various molecular species of ADA to dCF and EHNA [21]. It was found that the small-molecular-weight form of calf intestinal ADA was equally sensitive to both dCF and EHNA ($ID_{50} = 2 \times 10^{-11} M$), whereas the large-molecular-weight species or the crude mixture of ADA was less sensitive to EHNA vs dCF ($ID_{50} = 1 \times 10^{-7} M$ and $2 \times 10^{-8} M$ for EHNA and dCF, respectively, for the large-molecular-weight species, and $6 \times 10^{-7} M$ and $2 \times 10^{-8} M$, respectively, for the crude enzyme mixture). Striking differences in the sensitivity of ADA from various mouse and rat tissues to EHNA *in vitro* have also been noted; however, the reason for this variation was not elucidated with respect to the molecular species of ADA affected [19, 84].

In vivo studies examining the effect of continuous IV infusion of EHNA [19] and dCF [82] in mice have yielded striking results. EHNA infused at a dose of 4–40 mg/kg per day for 5 days increased ADA activity in the lung, colon, small intestine, stomach, spleen, liver and kidney, but decreased ADA activity in the thymus. In contrast, dCF infused at a dose of

0.4 mg/kg per day for 5 days severely inhibited ADA activity in the thymus, colon, lung, liver, and kidney, while not markedly affecting ADA activity in the spleen and small intestine, and elevating ADA activity in the stomach [82]. Interestingly, mice bearing a SC transplanted colon tumor showed a greater susceptibility to inhibition of ADA activity by dCF in the spleen, colon, and small intestine [82]. Thus, these results point to the necessity of slowly escalating the dose of dCF in patients with cancer, as well as the possible differences in response of reticuloendothelial tissues in normal vs cancer patients to dCF.

The kinetic parameters for inhibition by dCF in intact cells are quite different from cell-free or purified preparations of ADA. The association rate constant for intact erythrocytes or sarcoma 180 cells was 300- to 500-fold lower vs cell-free preparations [66]. This effect is probably the main determinant of the reduced ID_{50} of dCF for intracellular ADA, i.e., 10^{-8} to $10^{-7} M$ [33, 35]. On the other hand, the slower dissociation of the dCF-ADA complex in intact cells vs cell-free preparations [66] is undoubtedly a function of the rate of turnover of ADA in different cell types. This has recently been suggested by *in vivo* studies with dCF where the 48-h reactivation of ADA from inhibition by dCF was only 13% in erythrocytes which are anucleate and 80% in L1210 cells which are actively synthesizing mRNA [3, 19]. Additional considerations for the slow reactivation of intracellular ADA from inhibition by dCF are the varying sensitivities of different molecular species of

ADA [21, 71] and the presence of stabilizing or converting factors associated with ADA [23, 83].

The specificity of action of dCF and EHNA was studied in mouse lymphoma cells, where it was found that high concentrations (10^{-5} to 10^{-4} M range) of each drug affected all pathways of purine ribonucleotide synthesis [35]. Adenylate deaminase was also inhibited to a greater extent by dCF ($ID_{50} = 10^{-5}$ M) than by EHNA. Agarwal and Parks [5] found that coformycin and dCF, but not EHNA, inhibited muscle adenylate deaminase with a K_i of about 5×10^{-8} M and 2×10^{-8} M, respectively [5]. Recently, it was reported that the synthetic nucleotide analog of dCF, dCF-5'-monophosphate inhibited muscle adenylate deaminase with an ID_{50} of 2×10^{-7} M [28].

Potentiation of 2'-Deoxyadenosine and Adenosine Toxicity

One of the unique features of dCF is its high specificity for inhibiting only ADA with little if any effect on other cellular enzymes at optimal concentrations for inhibiting ADA [21]. Several laboratories have taken advantage of this unique pharmacological activity to study the cytotoxicological and biochemical effects of adenosine and 2'-deoxyadenosine when their deamination was prevented by the presence of dCF or another inhibitor of ADA.

The first indication that inhibition of deamination by adenosine could profoundly modify an intracellular process was reported by Wolberg et al. [90]. In the presence of EHNA and exogenous adenosine, the levels of cyclic AMP rose markedly in peritoneal lymphocytes sensitized to mouse ascites leukemia cells. Moreover, cytolysis of ascites cells by the sensitized lymphocytes was blocked by adenosine and this effect was enhanced 2- to 3-fold by the presence of EHNA. Increased elevations of cAMP in the presence of norepinephrine and dCF were also reported to occur in cerebral cortex slices *in vitro* [74].

The effects of ADA blockade on lymphocyte blastogenesis have been actively pursued since the study by Wolberg [90], in an attempt to assess the function of ADA in immunodeficiency. Since ADA activity does not increase during lymphocyte transformation [34, 65], its role is presumably a passive and protective one. It is known that most patients with combined immunodeficiency disease lack or contain low levels of ADA [20, 38, 71, 87]. This recessive trait is expressed by levels of 2'-dATP in lymphocytes and erythrocytes of these patients that

are 5- to 100-fold higher than normal [20, 26]. This genetic error has been mimicked pharmacologically with inhibitors of ADA. EHNA in the presence of adenosine inhibited concanavalin A-mediated stimulation of [^{3} H]leucine incorporation in human peripheral lymphocytes by 50%, but 500 times more EHNA was required to produce the same amount of inhibition of [^{3} H]thymidine incorporation [11]. In contrast, 2'-deoxyadenosine but not adenosine in the presence of 1×10^{-6} M dCF inhibited [^{3}]leucine incorporation during the first day of blastogenesis in phytohemagglutinin-stimulated lymphocytes [85]. dCF alone had only a marginal inhibitory effect on [^{3} H]leucine incorporation [85]. These results suggest that a pre-S-phase event is associated with 2'-deoxyadenosine toxicity and not simply inhibition of DNA synthesis, presumably via inhibition of ribonucleoside diphosphate reductase. Conversely, Harrap and Paine [34] reported that only DNA synthesis in mitogen-stimulated lymphocytes was affected by adenosine and coformycin, while DNA and protein synthesis in L1210 cells were equally affected by the same regimen. Nevertheless, the common denominator in these studies is that the cytotoxicity of adenosine or 2'-deoxyadenosine on lymphocyte blastogenesis or tumor cell growth is markedly potentiated by EHNA [11, 45, 73], coformycin [34], or dCF [45, 85], and that greater inhibition of lymphocyte transformation is produced by 2'-deoxyadenosine vs adenosine in the presence of dCF [45, 85] or EHNA [73].

Ullman et al. [86] showed that in a mouse lymphoma line lacking 2'-deoxyadenosine kinase, the sensitivity of these cells to EHNA was greatly diminished. This suggested that phosphorylation of 2'-deoxyadenosine is required for producing cytotoxicity, as found in the wild-type lymphoma cells which produced more than a 10-fold elevation in 2'-dATP in the presence of 10^{-5} M EHNA. In contrast, Hersfield et al. [37] reported that adenosine kinase-deficient human lymphoblasts remained as sensitive as the parent cell line to growth inhibition by adenosine in the presence of EHNA. This apparent divergence in mechanism of action between adenosine and 2'-deoxyadenosine indicates that adenosine-mediated cytotoxicity may be produced via an adenine nucleotide-independent process. The validity of this suggestion was shown in studies where the addition of exogenous adenosine in the presence of coformycin or dCF did not significantly elevate ATP levels in mouse leukemia cells [34, 50, 55] and mitogen-stimulated lymphocytes [34], but that 2'-deoxyadenosine in the presence of EHNA [86], dCF [50] or coformycin [34] produced moderate to high elevations of 2'-dATP.

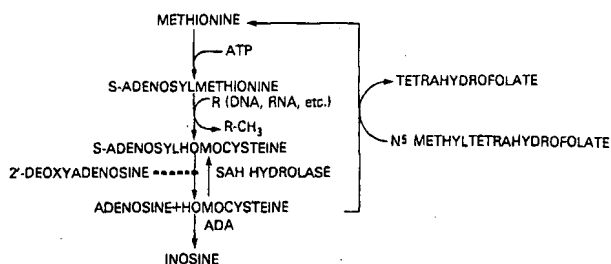


Fig. 3. Pathways for metabolism of S-adenosyl-L-methionine. Interference with hydrolysis of SAH via SAH hydrolase is denoted by the dashed line (---)

One essential metabolic process that may be directly affected by adenosine is methylation via S-adenosyl-L-methionine (Fig. 3). Kredich and Martin [43] and Kredich and Hershfield [42] showed that in the presence of EHNA and L-homocysteine thiolactone, adenosine produced marked toxicity to lymphoma cells and its effect correlated with the elevation of SAH and inhibition of DNA and RNA methylation. This mechanism for adenosine toxicity is particularly intriguing, since the reversibility of SAH hydrolase has been demonstrated *in vivo* [39] and SAH is a potent inhibitor of mRNA methylation and viral multiplication [63, 64]. Interestingly, the adenosine analog cordycepin (3'-deoxyadenosine) inhibits nuclear RNA methylation quite effectively [31] and may function via its conversion to 3'-deoxySAH [32], which is an effective inhibitor of 5'-cap methylation of mRNA [63, 64]. Zimmerman et al. [94] recently reported that human erythrocytes were capable of metabolizing cordycepin to an SAH-like metabolite in the presence of EHNA and homocysteine. Of equal importance was their observation that adenosine and homocysteine in the presence of EHNA could not only elevate SAH levels in lymphocytes but also increase the concentration of cyclic AMP [95]. This is particularly important, since both elevated levels of cyclic AMP and SAH could potentially inhibit, perhaps synergistically, normal lymphocyte function.

Alternatively, 2'-deoxyadenosine and adenine arabinoside have recently been reported to function as a tight-binding suicide inhibitor of SAH hydrolase causing irreversible inactivation of catalytic activity [36]. Such a phenomenon, if active *in vivo*, could indirectly elevate the intracellular concentrations of SAH, and thus produce impairment of methylation processes critical for cell growth and viability without invoking the necessity for the conversion of 2'-deoxyadenosine to its nucleotide form (Fig. 3). In recently completed studies, we have not found any evidence that 2'-deoxyadenosine in the presence of dCF can affect the intracellular concentration of SAH in

L1210 cells, although adenosine, homocysteine and dCF markedly elevated SAH levels and arrested the methylation of nuclear RNA [29a].

Potentiation of Adenosine Analogs

The expected synergism of ADA inhibitors with adenosine analogs possessing antitumor activity has been demonstrated in a number of tumor systems. When adenosine analogs were combined with EHNA, marked synergism with cordycepin and adenine arabinoside was seen in L cells in tissue culture and with the latter drug *in vivo* with Ehrlich ascites cells [60]. More recent experiments with dCF demonstrated marked potentiation by dCF of the cytotoxic effects of cordycepin, xylosyladenine, adenine arabinoside and other adenosine analogs in mouse lymphoid leukemia cells both *in vitro* and *in vivo* [1, 10-12, 40, 46, 49]. It is significant that the combination of dCF and adenine arabinoside prolonged the life-span of mice bearing intracerebrally implanted L1210 cells by 50%-90%, whereas either drug alone was inactive [10, 46]. dCF alone did not possess any antitumor activity.

It is presumed that the mechanism of this synergism is the sustained formation of high levels of the active nucleotide metabolite derived from the parent adenosine analog. This phenomenon has been corroborated by chromatography studies employing dCF and adenine arabinoside or xylosyladenine, where the 5'-triphosphate accumulates in the presence of dCF [16, 55, 59, 67]. High levels of the 5'-triphosphate of cordycepin and xylosyladenine would be expected to be markedly cytotoxic, and in fact are inhibitory to nuclear RNA synthesis in L1210 cells [33, 58]. The latter effects also quantitatively correlate with the degree of synergism seen with this two-drug combination and the inhibition of cell growth of mouse leukemia cells *in vitro* or the prolongation of life-span of mice bearing P388 leukemia [1, 40]. Interestingly, the effect of dCF on the inhibitory action of cordycepin on nRNA synthesis in regenerating rat liver, a rapid-dividing tissue, is only marginal due to the low level of ADA in this tissue [29]. Plunkett et al. [61] have recently reported that the main determinant of the effect of adenine arabinoside on DNA synthesis in mice bearing P388 leukemia and treated in addition with either EHNA or dCF is the retention of the active metabolite, AraATP, in the tumor cells. This effect occurred despite the more rapid excretion of EHNA and the less sustained inhibition of ADA by this drug in P388 cells in comparison to dCF [62]. In addition, the rephosphorylation and half-life of AraATP in CHO

cells was not influenced by the presence of either dCF or EHNA [72]. These data support the conclusion that the primary factor influencing the therapeutic activity of adenosine analogs such as adenine arabinoside when inhibitors of ADA are co-administered is the initial concentration of the active antitumor metabolite, and not the rate of excretion of the ADA inhibitor nor the absolute degree and length of inhibition of ADA in the tumor cell. This hypothesis is supported by the equal synergism that 1×10^{-6} M dCF or EHNA exert on the cytotoxicity of adenine arabinoside 5'-monophosphate in L1210 cells in culture [11].

Combinations of ADA inhibitors and adenine arabinoside also show pronounced antiviral activity. In initial investigations, it was reported that dCF and adenine arabinoside markedly inhibited the plaque-forming ability of herpes simplex I and II [8, 76, 89]. Similar effects were noted with the combination of adenine arabinoside with coformycin [77] and EHNA [57]. It is of interest that, whereas dCF alone had no antiviral activity [57, 76], EHNA inhibited the plaque-forming ability of herpes virus by 50% at 10^{-6} M [57]. The potentiation of the antiviral activity of cordycepin by EHNA also showed a direct correlation with inhibition of total RNA synthesis in the infected HeLa cells [57], a relationship that also parallels the effect that cordycepin and either dCF [32] or EHNA [59] have on mouse ascites tumor and fibroblast cells, respectively.

The antiviral activity of EHNA appears to be a characteristic of adenine analogs with aliphatic alcohol substituents at the 9-position [70]. It has recently been reported that 9-(2,3-dihydroxypropyl)adenine, an inhibitor of ADA, strongly inhibits herpes simplex I and II, as well as the RNA viruses that produce vesicular stomatitis and measles [25]. Other aliphatic alcohol analogs of adenine and guanine also possess antiviral activity [25], and it will be interesting to see whether these newer analogs also have potent inhibitory effects on ADA.

An interesting activity of the adenosine analogs cordycepin and xylosyladenine that is potentiated by dCF, is their ability to directly competitively inhibit the phosphorylation of nuclear proteins via cyclic nucleotide-independent protein kinases [30, 47, 48]. dCF potentiates the inhibitory effect of xylosyladenine on the phosphorylation of nonhistone chromosomal proteins in L1210 cells to a greater extent than cordycepin [48], a result quite opposite to the synergism seen with these drug combinations on RNA synthesis [33, 58]. Since the phosphorylation of nuclear proteins is believed to play an active role in regulating transcription [80] this synergistic effect of

dCF undoubtedly contributes to the overall interference by these drugs with transcription.

Immunosuppressive Activity

As EHNA can be distinguished from dCF by its intrinsic antiviral activity, so can dCF be differentiated by its highly selective immunosuppressive activity, which EHNA possesses to a lesser degree. Initial studies of the immunosuppressive effects of dCF used single doses of 0.5–1.0 mg/kg daily for 4 days, which inhibited ADA 80%–100% [9]. Inhibition of ADA in vivo under these conditions did not impair the mitogen-stimulated blastogenic response in vitro of thymocyte or spleen cells from these animals. However, this negative finding can be explained by the dilution of viable cells inhibited by dCF upon subculturing. When dCF is preincubated with lymphocyte cultures for 1–3 days before addition of the mitogen, it inhibits concanavalin A, phytohemagglutinin, and pokeweed mitogen-mediated blastogenesis in human peripheral lymphocytes with an ID_{50} of approximately 1×10^{-6} M [18]. In the latter experiments, blastogenesis was measured by [^{3} H]thymidine incorporation 3 days after the addition of mitogen. In studies where dCF was added simultaneously with the mitogen to lymphocyte cultures, and blastogenesis was assessed by [^{3} H]leucine incorporation at 1 day after addition of mitogen, only 15% inhibition was produced by 1×10^{-6} M dCF [85]. These studies suggest that the immunosuppressive action of dCF is associated with DNA synthesis or at least some biosynthetic event occurring during S-phase.

Immunosuppressive activity by dCF and to a lesser extent by EHNA was demonstrated by allograft acceptance of LSTRA tumor cells across the H-2 histocompatibility locus [2, 17]. A single dose of 7.5–15 mg dCF/kg administered 24 h before the transplant resulted in 53% on 68% allograft acceptance vs 19% acceptance by a similar regimen of 200 mg EHNA/kg. Divided doses of 0.25–0.5 mg dCF/kg or 150 mg EHNA/kg daily for 6 days only produced 20% and 10% acceptance, respectively. EHNA administered at 10 mg/kg daily for 14 days produced a 60% acceptance rate of mouse pancreatic islet allografts across a non-H-2 histocompatibility barrier [52]. Skin graft survival in mice across a non-H-2 histocompatibility barrier was also markedly prolonged (73%–83% graft survival) by 18 daily doses of 3.3 and 50 mg EHNA/kg [51]. Thus, dCF appears to be a more potent immunosuppressive drug against a strong antigenic barrier, whereas EHNA although

not devoid of immunosuppressive activity appears to be a less effective agent.

The selectivity of dCF for lymphoid cells in mice [78] has also been confirmed in cancer patients where predominantly lymphocytopenia has been observed [41, 79].

The biochemical effects of EHNA on nucleotide and RNA synthesis have been examined in phytohemagglutinin-stimulated lymphocytes [75]. At concentrations of $1 \times 10^{-5} M$, which completely inhibit ADA, EHNA inhibited the phosphoribosylpyrophosphate-dependent formation of ATP and ITP from adenine and hypoxanthine, respectively. Ribonucleotide pool sizes and RNA synthesis were not affected except at exceedingly high concentrations of $5 \times 10^{-4} M$. These results are consonant with the study by Henderson et al. [35], who also found that high concentrations of EHNA, as well as dCF, inhibited ribonucleotide biosynthesis in mouse lymphoma cells. It should be noted that these ancillary activities are achieved at concentrations of inhibitor that are 10- to 100-fold greater than necessary to inhibit ADA. They may be related, however, to the immunosuppressive effects of these drugs, which are produced only at dosages much greater than that required for inhibiting only ADA.

Disposition, Excretion and Metabolism

The ADA inhibitor most extensively studied pharmacokinetically has been dCF. The tight-binding characteristics of dCF have permitted the development of a highly specific enzymatic assay, which measures the inhibition of ADA in the presence of biological fluids. With these techniques, it has been shown that the distribution of dCF closely follows the concentration of ADA in the various tissues of all species of animals studied thus far [7, 19, 53].

The plasma $t_{1/2}$ for the dCF between rodents and dogs is similar (Table 1). dCF is rapidly cleared in the urine, but the rate appears to be much slower in man [79]. The latter effect is explained by the longer plasma $t_{1/2}$ in man which was not predicted by the preclinical data. The recent reports by Suling et al. [81] and Plunkett et al. [62] suggest that the duration of action of EHNA is approximately 50% of dCF as judged by their respective effects on increasing the $t_{1/2}$ of adenine arabinoside in the plasma [81] and ascites fluid [62] in mice. Approximately 50% of an IV dose of 5 mg EHNA/kg was cleared from the plasma in 1 h in the Rhesus monkey [54].

The total cumulative excretion of dCF in the urine and feces was 90%–100% of the administered dose of dCF [7, 19, 53]. Recently, studies of the metabolism

Table 1. Urinary excretion and plasma $t_{1/2}$ for dCF

Species	Plasma $t_{1/2}$ (min)		% Recovered unchanged in urine in 24 h	Reference
	α phase	β phase		
Mouse	17–19	64–104	100	[53]
Rat	—	—	80	[7]
Dog	12–15	90–120	77	[19]
Man	45–90	240–360	34	[79]

of dCF in L1210 cells in vitro have demonstrated that only 10%–15% of the drug (10^{-6} to $10^{-4} M$) is converted to the nucleoside monophosphate in 1–2 h, but not further anabolized [88]. This is in contrast to the report by Muller et al. [55] that in mouse L5178Y leukemia cells in culture, 54% of $1 \times 10^{-6} M$ coformycin is recovered as mono-, di- and triphosphates upon exposure to the drug for 24 h. EHNA is almost completely metabolized in mice with numerous hydroxylated metabolites of the aliphatic hydrocarbon moiety being present in the urine after IP administration [44]. This finding has been confirmed in the Rhesus monkey, where 70% of the administered IV dose was eliminated in the urine after 6 h, almost entirely as four metabolites [54].

Conclusions

This review has emphasized the pharmacological activities of ADA inhibitors, both as single agents and in combination chemotherapy. The two most studied drugs, EHNA and dCF, each have some unique attributes that should allow for them to be further developed on their own merits for clinical trials. Both drugs produce little or no effect on other processes associated with de novo purine synthesis or adenine nucleotide catabolism at concentrations that completely inhibit ADA; however, at 100-fold higher concentrations, EHNA appears less specific with respect to its site of action than dCF [35].

EHNA possesses antiviral activity, which dCF does not, and thus would be expected to be more efficacious in combination with antiviral adenosine analogs in the chemotherapy of acute viral infections. The development of closely related aliphatic alcohol analogs of adenine with potent antiviral activity attests to the unique properties of this structural class of drugs. On the other hand, dCF would be more advantageous for the chemotherapeutic treatment of cancer in combination with adenosine analogs, or quite possibly as a single agent in lymphoid leukemias. The longer half-life of dCF and its more

prolonged and potent inhibitory effects on ADA vs EHNA would be a particularly favorable pharmacological property for the chronic treatment of cancer patients. The intrinsic immunosuppressive activity of dCF at higher doses is an added feature of this drug. It may serve as a prototype for a new class of immunosuppressive agents lacking many of the unfavorable side effects of the current modalities of immunosuppressive therapy.

From an experimentalist's perspective, the use of ADA inhibitors has helped enormously in understanding the mechanism of action of adenosine metabolites and analogs whose catabolism was heretofore neglected with respect to their specificities of action. ADA inhibitors have also enabled us to understand the regulatory processes associated with immunodeficiencies characterized by a lack of ADA, and to further understand the maturation of the immune response.

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Received November 20, 1979/Accepted June 4, 1980