

**SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
DIRECCIÓN DE NUEVAS CREACIONES**

2020
Bogotá, D.C.,

Abogado
LUIS PATIÑO LEYVA.
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ASUNTO	Radicación	09-22669	13986
	Trámite	011	
	Evento	378	
	Actuación	519	
	Oficio		
	Folios	008	

Patente de Invención	☐	Patente de Modelo de Utilidad	☐
Vía Nacional	☐		
Vía PCT (fase nacional)	☐	Cap. I ☐	Cap. II ☐

No. Solicitud Internacional PCT/US2007/017463
Fecha de Presentación Internacional 06 de agosto de 2007.

Como resultado del Examen de Fondo realizado, con fundamento en el Artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se comunica:

1. Documentos estudiados

Descripción:	Folios 114 a 264	05/marzo/2009
	Folios 336 a 524	07/junio/2009
Reivindicaciones:	Folios 265 a 274	05/marzo/2009
	Folios 532 a 548	11/octubre/2010
Prioridad:	US 60836158	08/agosto/2006

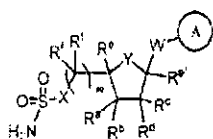
2. Análisis de la Prioridad

Se acepta la reivindicación de prioridad porque: esta solicitud fue presentada dentro del plazo establecido (Art. 9 D 486) y su contenido técnico corresponde con el de la prioridad reivindicada.

3. Objeto de la invención

Título de la solicitud: "Compuesto de heteroarilo útiles como inhibidores de enzimas de activación"

El problema planteado en esta solicitud consiste en suministrar proporcionar compuestos útiles para desórdenes de proliferación celular como cáncer y



Compuesto I

El objeto de la presente solicitud se relaciona con la Clasificación Internacional de Patentes (CIP): A61K 31/505, A61P 35/00 y C07D 213/82 // 239/48 // 251/18 // 403/04 // 403/08 // 473/32 // 498/04

4. Excepción a la Patentabilidad (Art 20 D 486 literales (a) – (d))

El objeto de las reivindicaciones 25-27 no es patentable de acuerdo con el Art citado.

5. De la Solicitud de Patente

Reivindicaciones (Art 30 D 486, Art 22 PCT)

Las reivindicaciones 1 – 41 no son concisas porque de las definiciones de $R^a, R^b, R^c, R^d, R^e, R^f, R^g, R^h, R^i, R^j, R^k, W, D, E, F, G, N, Q, U, X, Y, Z, V^1, T^1, R^1, R^m, R^n, R^o, R^p, R^q, R^r, R^s, R^t, R^u, R^v, R^w, R^x, R^y, R^z, R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9, R^{10}, R^{11}, R^{12}, R^{13}, R^{14}, R^{15}, R^{16}, R^{17}, R^{18}, R^{19}, R^{20}, R^{21}, R^{22}, R^{23}, R^{24}, R^{25}, R^{26}, R^{27}, R^{28}, R^{29}, R^{30}, R^{31}, R^{32}, R^{33}, R^{34}, R^{35}, R^{36}, R^{37}, R^{38}, R^{39}, R^{40}, R^{41}, R^{42}, R^{43}, R^{44}, R^{45}, R^{46}, R^{47}, R^{48}, R^{49}, R^{50}, R^{51}, R^{52}, R^{53}, R^{54}, R^{55}, R^{56}, R^{57}, R^{58}, R^{59}, R^{60}, R^{61}, R^{62}, R^{63}, R^{64}, R^{65}, R^{66}, R^{67}, R^{68}, R^{69}, R^{70}, R^{71}, R^{72}, R^{73}, R^{74}, R^{75}, R^{76}, R^{77}, R^{78}, R^{79}, R^{80}, R^{81}, R^{82}, R^{83}, R^{84}, R^{85}, R^{86}, R^{87}, R^{88}, R^{89}, R^{90}, R^{91}, R^{92}, R^{93}, R^{94}, R^{95}, R^{96}, R^{97}, R^{98}, R^{99}, R^{100}$ se obtiene un gran número de compuestos, muchos de los cuales no están ejemplificados en la forma de ejemplos de la invención dentro del capítulo descriptivo, se sugiere restringir la solicitud a una razonable generalización del tipo de compuestos que han sido sintetizados o probados, es decir, que se encuentran debidamente sustentados. El siguiente cuadro ejemplifica la afirmación anterior y presenta los sustituyentes que se encuentran soportados en la descripción.

Sustituyentes de acuerdo con la reivindicación 1 de la solicitud		Sustituyentes soportados en la descripción
R^a y R^c	hidrógeno, fluoro, CN, $-N_3$, $-OR^5$, $-N(R^4)_2$, $-NR^4CO_2R^6$, $-NR^4C(O)R^5$, $-C(O)N(R^4)_2$, $-C(O)R^5$, $-OC(O)N(R^4)_2$, $-OC(O)R^5$, $-OCO_2R^6$, o un C_{1-4} alifático o C_{1-4} fluoroalifático opcionalmente sustituido con un grupo seleccionado de $-OR^{5a}$, $-N(R^{4x})(R^{4y})$, $-CO_2R^{5a}$, o $-C(O)N(R^4)(R^{4*})$; o R^a y R^c junto forman un enlace;	Hidrógeno y Hidroxilo
R^b y R^d	hidrógeno, fluoro, C_{1-4} alifático o C_{1-4} fluoroalifático	Hidrógeno y Hidroxilo
R^f	hidrógeno, fluoro, un C_{1-4} alifático o C_{1-4} fluoroalifático o dos R^f tomados en forma conjunta forman $=O$, dos R^f tomados en forma conjunta con el átomo de carbono al que están unidos, forman un anillo carbocíclico de 3-6 miembros; o R^f , tomado en forma conjunta con R^e y los átomos de carbono intervinientes forma un espirociclo de 3-6 miembros	Hidrógeno
W	$-CH_2-$, $-CHF-$, $-CF_2-$, $-CH(R^1)-$, $-CF(R^1)-$, $-NH-$, $-N(R^1)-$, $-O-$, $-S-$, o $-NHC(O)-$	$-CH_2-$, $-O-$, $-NH-$ y $-N(R^1)$,
X	$-CH_2-$, $-CHF-$, $-CF_2-$, $-NH-$ y $-O-$	$-NH-$ y $-O-$
Y	$-O-$, $-S-$ o $-C(R^m)(R^n)$	$-C(R^m)(R^n)$

La reivindicación 37 no es clara porque refiere ser dependiente de la reivindicación 40, sin embargo, la reivindicación 40 simultáneamente refiere ser dependiente de la reivindicación 37 .

Descripción (Art 28 D 486)

La Descripción no es clara ni concisa porque la utilización de las expresiones (fol. 341) “y otros desórdenes asociados con la actividad de E1” y que (Fol. 341) “poner como blanco enzimas de activación E1 provee una oportunidad único de interferir con un variedad de importantes vías bioquímicas para mantener la integridad de la división celular y de la señalización celular” refiriéndose a las posibles actividades farmacológicas del compuesto no permite que la persona del oficio normalmente versada en la materia identifique o restrinja el ámbito de aplicación de la invención.

La descripción no clara porque no permite a una persona versada en la materia identificar un único concepto inventivo, dado que como lo manifiesta el solicitante a través de sus realizaciones preferidas I-110 y I-138 (Fol. 388 y 392) estos compuestos son inhibidores de la enzima de activación E1 pero no comparten los elementos técnicos esenciales de la invención.

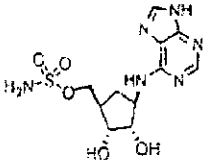
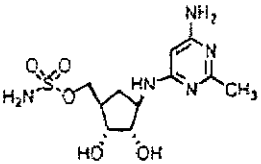
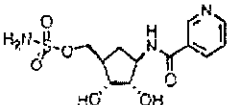
6. Unidad de invención (Art 25 D 486)

La Solicitud no cumple con el Requisito de Unidad de Invención porque se refiere a por lo menos 3, Grupos Inventivos de acuerdo con las realizaciones preferidas presentadas.

Los Grupos Inventivos están definidos por las siguientes características esenciales:

- 1° Compuestos en los cuales el núcleo está caracterizado por ser derivados del sulfamato del [(1*R*,2*R*,3*R*,4*R*)-4-[(9*H*-purin-6-amino ó oxo-il)ciclopentil]metilo.
- 2° Compuestos en los cuales el núcleo está caracterizado por ser derivados del sulfamato del [(1*R*,2*R*,3*R*,4*R*)-4-[(piridin ó pirimidin ó 1,3,5-triazina-((2 ó 4-amino ó oxo-il)ciclopentil]metilo
- 3° Compuestos en los cuales el núcleo está caracterizado por ser derivados del sulfamato del [(1*R*,2*R*,3*R*,4*R*)-4-{piridin-2-ilcarbonil)-amino}ciclopentil]metilo.

Los grupos Inventivos de acuerdo con las realizaciones preferidas, son descritos a continuación:

Grupo inventivo	Característica esencial de grupo inventivo (estructura química central)	Realizaciones preferida del grupo inventivo
1	 Compuesto I-3	3, 9, 34, 36-39, 41, 49, 56, 62-64, 67-70, 74, 80, 84, 87, 90, 93, 99-105, 107, 109,111,112, 117, 121-123, 126, 128-131, 137-140, 146 y 152.
2	 Compuesto I-13	1-2, 4-8, 10-15, 17-22, 24-33, 35, 40, 42-48, 50-55, 57-61, 65, 66, 71-73, 75-79, 81-83, 85, 86, 88, 89, 91, 92, 94-98,106,108,113-116,118-120,124-125,127,132-136,141-145,147-151 y 153.
3	 Compuesto I-16	16 y 23.

La evaluación de la unidad de invención para esta solicitud fue efectuada a través del método problema-solución en forma a posteriori y se presenta en el examen de patentabilidad efectuado al primer grupo inventivo, encontrándose que los documentos D1 y D2 anticipan el concepto común inventivo de la solicitud. Adicionalmente, se reitera que la solicitud presenta una estructura anular hipervariable del tipo A-B-C-D en lugar de una estructura central química definida. En consecuencia, la solicitud no cumple el requisito del Artículo 25 de la Decisión 486 de 2000 de la Comisión de la Comunidad Andina de Naciones. Este despacho sugiere presentar las solicitudes divisionales que considere necesarias, ó limitar la solicitud inicial a una sola invención, eliminando de ella las otras invenciones.

7. Determinación del Estado de la Técnica

El estado de la técnica se determinó con base en la fecha de presentación de la solicitud de prioridad agosto 8 de 2006 y se encontraron los siguientes documentos que anticipan el objeto de esta solicitud:

Ítem	Documento	Título	Fecha de Publicación*
D1	J. Med. Chem. 1992,35, 3991-4000	Synthesis and Biological Evaluation of 5'-Sulfamoylated Purinyl Carbocyclic Nucleosides	1992
D2	Bioorganic & Medicinal Chemistry Letters 15 (2005) 3389-3393	Deoxyribosyl analogues of methionyl and isoleucyl sulfamate adenylates as inhibitors of methionyl-tRNA and isoleucyl-tRNA synthetases	2005

D1: Es un estudio que divulga (Abs.) los resultados de una serie de nucleósidos purinil carbocíclico 5'-sulfamoilados que fueron sintetizados y evaluados en su actividad antitumoral y antibacteriana.

Adicionalmente, divulga (Pág. 1, Col. 1) que muchos análogos de la nucleocidina defluorinados que contienen 5'sulfamoil grupo han sido evaluados y estos agentes muestran actividad antibacterial, antitumoral, antiviral, antiparasitaria y herbicida.

De igual modo, indica (Pág. 1, Col. 2) que cuando se sustituye el en anillo del azúcar del nucleósido, el átomo de Oxígeno por un radical metileno, se obtienen derivados de estructura muy similar a los naturales y que los productos mono, di o trifosforilados formados en estas reacciones son con frecuencia agentes antivirales y antitumorales.

También, Informa (Pág. 1, Col. 1) que todos los agentes seleccionados para este estudio contienen una adenina o un base similar a adenina y que esta selección fue basada en los resultados del estudio de estudio de Bloch y Coutsogeorgopoulos, donde también se incluían análogos defluorinados de la nucleocidina que contenían adenina, inosina, citidina, uracilo y timidina.

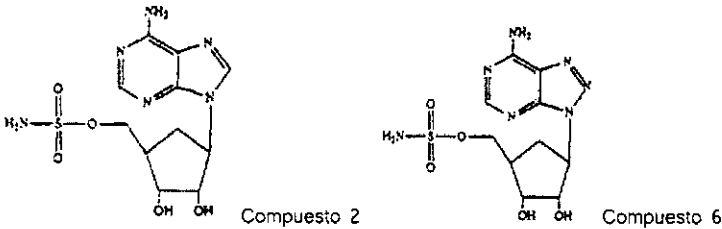
Por otra parte, da a conocer en la tabla 1 (Pág 3, Col. 2) que los resultados de los estudios de actividad de los compuestos, indicando que los compuestos 2 y 6 fueron agentes citotóxicos muy potentes y que los otros nuevos agentes 4 y 5, exhiben alguna toxicidad pero significativamente menor que la presentada por los compuestos 2 y 6.

Table I. Cytotoxicity to P388 Mouse Leukemia Cells

Nc1ncnc2c1ncn2[C@H]3O[C@H](COP(=O)(O)O)[C@@H](O)[C@H]3O

	X	Z	Y	IC ₅₀ (μg/mL)
2	NH ₂	CH	OSO ₂ NH ₂	0.062-0.125
3	NHCH ₃	CH	OSO ₂ NH ₂	2-4
4	N(CH ₃) ₂	CH	OSO ₂ NH ₂	~74
5	SCH ₃	CH	OSO ₂ NH ₂	~50
6	NH ₂	N	OSO ₂ NH ₂	0.0156-0.0625
8 (acyclo)	NH ₂	CH	OSO ₂ NH ₂	>100
C-Ado	NH ₂	CH	OH	~0.5
17	NH ₂	N	OH	0.25-0.5
puromycin	-	-	-	0.25-0.5

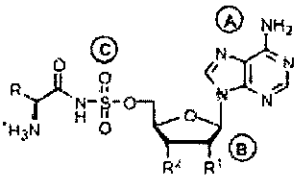
Para concluir afirma que los compuestos 2 y 6 fueron evaluados frente a conocidos agentes antitumorales con C-Ado y 8-aza-adenosina carbocíclica y que los resultados muestran que los compuesto 2 y 6 son significativamente más potentes y finalmente, indica (Pág. 5, Col.1) que las 5′sulfamoiladenosinas son inhibidores de la formación de los aminoacil-tRNA’s.



D2: divulga [Abstract] que los compuestos metionil y isoleucil sulfamatos adenilatos son inhibidores de la metionil-tRNA y isoleucil-tRNA sintetasas entre otras tRNA sintetasas respectivamente, las cuales corresponden a una familia de enzimas encargadas precisamente de la transferencia de aminoácidos del tRNA para formar aminoacil-tRNAs, los cuales son sustratos para la traducción en la síntesis de proteínas.

De igual forma, enseña (Pág. 101 Col. 2) que se han realizado múltiples investigaciones sobre las modificaciones de los aminoacil adenilato con el propósito de mejorar o proveer estabilidad química entre otras y se ha observado que consistentemente que los sulfamatos análogos muestran la mayor potencia de inhibición de la enzimas.

En otro aparte, indica que (Pág. 2 ,Col. 1) y (Pág. 2, Fig. 1) las modificaciones a este grupo de moléculas han estado enfocadas a tres regiones farmacofóricas que incluye los grupos adenina, ribosa y acil fosfato.



Finalmente, divulga que el efecto inhibitorio de los derivados 2′,3′-dideoxiribosil es mucho más potente que los derivados 2′ ó 3′-deoxiribosil solos.

8. Examen de Patentabilidad (Art 14 D486)

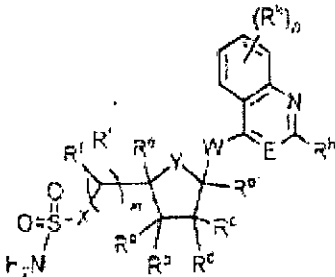
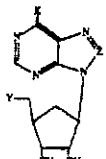
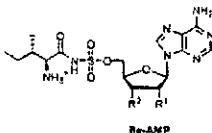
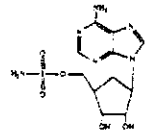
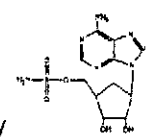
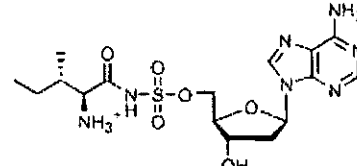
Nivel Inventivo (Art18 D486)

modificaría el compuesto divulgado en D1 sustituyendo la adenina por un base similar a adenina, es decir un bioisómero ó análogos defluorinados de la nucleocidina que contengan adenina ó inosina ó citidina ó uracilo ó timidina de acuerdo con lo divulgado por D1, para así llegar al objeto de la invención. Por lo tanto la reivindicación 1 se considera obvia.

Dado que las reivindicaciones 2, 3, 5 y 15 a 17 no mencionan características técnicas esenciales, éstas tampoco se puede considerar inventivas.

Teniendo en cuenta la falta de unidad de invención, se elige el primer grupo inventivo para estudiar la patentabilidad de la invención para la reivindicación 22 caracterizado por ser un compuesto derivados del sulfamato del [(1*R*,2*R*,3*R*,4*R*)-4-[(quinoleína ó quinazolina-6-amino ó oxo-il)ciclopentil]metilo.

Comparación de las características técnicas **esenciales**, con las del Estado de la Técnica:

Características esenciales	D1	D2
Compuesto VII	El compuesto 1	Una serie de compuesto de fórmula
	 X Z Y NH₂ C Un grupo Sulfamoi	 Be-AMP
X O Y C o O W NH₂ E NH₂ R H R^K H	El compuesto 2 y 6	El compuesto 2
	 compuesto 2 y  compuesto 6	
	Indica que los agentes seleccionados para este estudio todos contienen una adenina o un base similar a adenina y que esta selección fue basada en los resultados del estudio de Bloch y Coutsoegeorgopoulos, donde también se incluían análogos defluorinados de la nucleocidina que contenían adenina, inosina, citidina, uracilo y timidina.	Las modificaciones a este grupo de moléculas han estado enfocadas a tres regiones farmacofóricas que incluye los grupos adenina, ribosa y acil fosfato.

ciclopentil y no una ribosa como lo indica el documento D2

El efecto que logra el modificar una purina por una quinoleina ó quinazolina, es obtener nuevos compuesto inhibidores de la Enzima de activación E1 con actividad antitumoral.

Por lo tanto, el Problema Técnico Objetivo que pretende resolver esta invención se puede formular así: "cómo modificar los compuestos con actividad antitumoral descritos en D1 para así obtener compuesto alternativos inhibidores de la enzima de activación E1 y por lo tanto útiles en el tratamiento de enfermedades antiproliferativas.

Sin embargo, el documento D2 divulga que las modificaciones a este grupo de moléculas han estado enfocadas a tres regiones farmacofóricas que incluye los grupos adenina, ribosa y acil fosfato y el documento D1 muestra como la introducción de un grupo bioisostérico de la adenina como un derivado 3H-[1,2,3]triazolo[4,5-d]pirimidina no afecta la actividad actitumoral de los compuesto objeto de la invención.

En consecuencia, la persona del oficio normalmente versada en la materia modificaría el compuesto divulgado en D1 sustituyendo la adenina una serie bioisotérica como esta sugerido en el documento D2 de acuerdo con lo divulgado por D1, para así llegar al Objeto de la invención. Por lo tanto la reivindicación 22 se considera obvia.

9. Conclusión

Los requisitos de Patentabilidad de la presente Solicitud están afectados así:

Ítem	Documento	Reivindicaciones afectadas	Requisito que afecta
D1	J. Med. Chem. 1992,35, 3991-4000	1-3, 5,15-17 y 22	Nivel inventivo
D2	Bioorganic & Medicinal Chemistry Letters 15 (2005) 3389-3393	22	Nivel inventivo

En cumplimiento del artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se le indica que debe dar respuesta dentro del término de sesenta (60) días contados a partir de la fecha de notificación de la presente comunicación. En caso de no dar respuesta al presente requerimiento, o si a pesar de la respuesta subsistieran los impedimentos para la concesión, se denegará la patente.

Se invita al solicitante se sirva indicar, si así lo considera, su renuncia al término faltante (60 días) y presentarla por escrito.

Notifíquese el presente oficio conforme a lo dispuesto en el numeral 5.2, literal e), del capítulo quinto del título primero de la Circular Única No. 10 de 2001.


JOSÉ LUIS SALAZAR LÓPEZ
 Director de Nuevas Creaciones (C)

14 AGO. 2012

El anterior requerimiento fue notificado por fijación en lista, de fecha _____

Elaborado por: Lenin Roberto Guerrero. 
 Revisado Por: Consuelo Leguizamón Leguizamón.
 Aprobado por: José Luis Salazar López.

Expediente 09-22669 Artículo 45

Synthesis and Biological Evaluation of 5'-Sulfamoylated Purinyl Carbocyclic Nucleosides

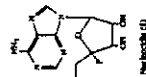
Fileen M. Peterson, Jay Brownell, and Robert Vince*

Department of Medicinal Chemistry, College of Pharmacy, 398 Harvard Street S.E., University of Minnesota, Minneapolis, Minnesota 55455-0343

Received July 2, 1992

The first series of 5'-sulfamoylated carbocyclic purinyl nucleosides was synthesized and tested for antitumor and antibacterial activities. The target compounds were formed by reacting the 2',3'-acetonide-protected carbocyclic nucleosides with sulfamoyl chloride, followed by deprotection. The agents were tested for cytotoxic activity against P-388 mouse leukemia cells. Two compounds, 5'-sulfamoyl carbocyclic adenosine (2) and 5'-sulfamoyl-8-aza carbocyclic adenosine (6) exhibited IC₅₀ values as low as 62 and 15 nM, respectively. These analogs inhibited protein biosynthesis and slowed down DNA and RNA biosynthesis in the P-388 cells. None of the target molecules were as potent against *Escherichia coli* as they were against the tumor cells. Also, in cell-free systems, agents 2 and 6 were more effective inhibitors of protein synthesis in rabbit reticulocyte lysate than in *E. coli*. These new carbocyclic derivatives appear to be somewhat selective for eukaryotic over prokaryotic cells in affecting translation.

Nucleoside (1), a nucleoside antibiotic produced by the bacteria *Streptomyces celvius*,^{1,2} is active against a wide variety of Gram-positive bacteria, gram-negative bacteria and trypanosomes.³ However, its high toxicity has prevented its therapeutic use. The structure of nucleoside (1) differs from the structure of other natural nucleosides in that it contains 4'-fluoro and 5'-sulfamoyl groups.⁴ Since the discovery of nucleoside (1), many defluorinated analogs containing the 5'-sulfamoyl group have been examined. These agents have been shown to exhibit antibacterial, antitumor,^{5,6} antiviral,^{7,8,9,10} antiparasitic,^{11,12} and herbicidal¹³ activities.



In this study, the first series of carbocyclic 5'-sulfamoylated nucleosides is reported, and the new agents are evaluated for antibacterial and antitumor activities. Carbocyclic nucleosides, nucleosides in which the sugar ring oxygen has been replaced with a methylene group, are very similar in structure to the natural derivatives; thus, many carbocyclic agents are recognized by nucleoside and nucleoside kinases and are subsequently phosphorylated.¹⁴ The mono-, di-, and triphosphorylated products formed in these reactions are often effective antitumor and antiviral agents.¹⁵ Carbocyclic adenosine (C-Ado), for example, forms a monophosphate capable of inhibiting at least one enzyme in purine de novo biosynthesis.¹⁷ This

- (13) Cammarosa, M. J.; Fernandez-Ram, P.; Garcia-Lopez, M. T.; de la Haza, P. G.; Mendes-Carvalho, P. P.; Alvarez, B.; Carrasco, L.; Urdiales, J. C. *Anticancer Res.* 1991, 11, 1071-1074.
- (14) Huet, D. L.; Schreier, S.; Allen, P. W.; Bennett, Jr., L. L. *Inhibition of Purine Metabolism in Mammalian Tumor Cells by the Carbocyclic Analogs of Adenosine*. *Mol. Pharmacol.* 1971, 7, 375-380.
- (15) Schreier, S.; Allen, P. W.; Bennett, Jr., L. L. *Specificity of Purine Nucleoside Phosphorylation by Tumor Cells*. *Colloid. Carcin. Chem. Commun.* 1971, 42, 370-381.
- (16) Bennett, Jr., L. L.; Allen, P. W.; Huet, D. L. *Metabolic Studies with Carbocyclic Analogs of Purine Nucleosides*. *Mol. Pharmacol.* 1965, 4, 200-217.
- (17) Schreier, S.; Allen, P. W.; Bennett, Jr., L. L. *The Synthesis of Adenosine 3'-O-Sulfamoyl Nucleosides Related to Nucleoside (1)*. *J. Am. Chem. Soc.* 1969, 91, 3391-3392.
- (18) Schreier, S.; Allen, P. W.; Bennett, Jr., L. L. *The Synthesis of Carbocyclic Analogs of Purine Nucleosides Related to Nucleoside (1)*. *J. Am. Chem. Soc.* 1970, 92, 3434-3440.
- (19) Schreier, S.; Allen, P. W.; Bennett, Jr., L. L. *The Synthesis of Carbocyclic Analogs of Purine Nucleosides Related to Nucleoside (1)*. *J. Am. Chem. Soc.* 1971, 93, 4320-4324.
- (20) Schreier, S.; Allen, P. W.; Bennett, Jr., L. L. *The Synthesis of Carbocyclic Analogs of Purine Nucleosides Related to Nucleoside (1)*. *J. Am. Chem. Soc.* 1972, 94, 3346-3357.
- (21) Schreier, S.; Allen, P. W.; Bennett, Jr., L. L. *The Synthesis of Carbocyclic Analogs of Purine Nucleosides Related to Nucleoside (1)*. *J. Am. Chem. Soc.* 1973, 95, 307-308.

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inhibition may contribute to C-Ado's in vitro antitumor activity. Carbocyclic nucleosides also have metabolic features which make them attractive for in vivo use. These compounds are resistant to the actions of purine nucleoside phosphorylases (PNPases),¹⁸ and they are poor substrates for natural nucleoside for adenosine deaminase (ADA's).^{19,20} Because of this resistance to normal purine metabolism, carbocyclic nucleosides may have longer in vivo half-lives than natural sugar nucleosides and therefore could yield drugs with longer durations of action.

The carbocyclic agents chosen for this investigation all contain an adenine or adenine-like base. This choice was based on the results of a study by Blinich and Coutage²¹ in which they demonstrated that the carbocyclic nucleoside containing adenine, inosine, cytidine, uracil, and thymine as the bases attached to a 5'-sulfamoylated sugar ring were necessary for antibacterial and antitumor activities. Thus, the target compounds all consist of an adenine (2) or adenine-like (3-6) base combined with a 5'-sulfamoylated carbocyclic sugar ring (Table I). The 6-position of purine ring in these compounds was explored by replacing the amino group with N-methyl (3), N,N-dimethyl (4), and S-methyl (5) groups. An 8-azadenine base (6) was also attached to the carbocyclic ring since 8-azapurines have often exhibited greater antitumor activity than the natural purine agents.¹⁶ Of the new agents studied, two (2 and 6) displayed extremely potent antitumor activity, and therefore preliminary studies on their mechanisms of action were performed.

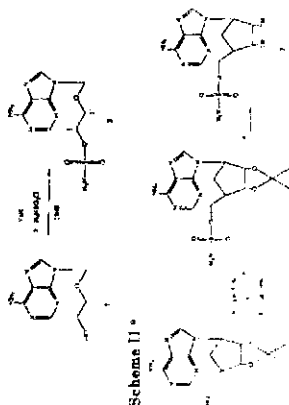
Chemistry

The general synthetic procedure involved the reaction of sulfamoyl chloride with 2',3'-acetonide-protected carbocyclic nucleosides followed by deprotection. In the synthesis of 5'-sulfamoyl nucleosides by Shuman et al.,²² the sulfamoylation was effected by activation of the 5'-hydroxyl on the orthoester-protected nucleoside with sodium hydride followed by reaction with sulfamoyl chloride. In the synthesis of nucleoside (1), Jenkins et al. activated the acetonide-protected nucleoside by reacting with bis(tributyltin)oxide and followed with the addition of sulfamoyl chloride.²³

As a model reaction, acyladenosine (7) originally reported by Schaeffer et al.,²⁴ was reacted with sodium hydride and sulfamoyl chloride, and this produced 9-(2-sulfamoyl-9-oxo-9H-purin-6-yl)methyladenosine (8) (Scheme 1). Likewise, when 2',3'-isopropylidene carbocyclic adenosine

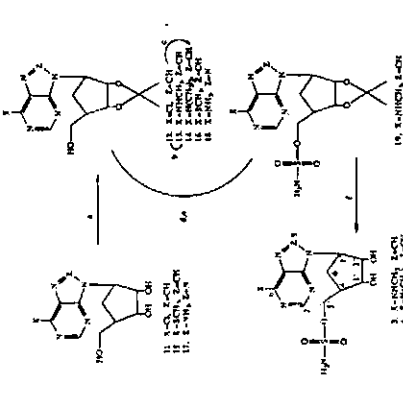
- (17) Huet, D. L.; Schreier, S.; Allen, P. W.; Bennett, Jr., L. L. *Inhibition of Purine Metabolism in Mammalian Tumor Cells by the Carbocyclic Analogs of Adenosine*. *Mol. Pharmacol.* 1971, 7, 375-380.
- (18) Schreier, S.; Allen, P. W.; Bennett, Jr., L. L. *Specificity of Purine Nucleoside Phosphorylation by Tumor Cells*. *Colloid. Carcin. Chem. Commun.* 1971, 42, 370-381.
- (19) Bennett, Jr., L. L.; Allen, P. W.; Huet, D. L. *Metabolic Studies with Carbocyclic Analogs of Purine Nucleosides*. *Mol. Pharmacol.* 1965, 4, 200-217.
- (20) Schreier, S.; Allen, P. W.; Bennett, Jr., L. L. *The Synthesis of Adenosine 3'-O-Sulfamoyl Nucleosides Related to Nucleoside (1)*. *J. Am. Chem. Soc.* 1969, 91, 3391-3392.
- (21) Blinich, R. K.; Coutage, D. A.; Robinson, M. J.; Robinson, M. J.; Robinson, R. K. *The Synthesis of Nucleoside Sulfamoyl Analogs Related to Nucleoside (1)*. *J. Am. Chem. Soc.* 1970, 92, 3434-3440.
- (22) Schreier, S.; Allen, P. W.; Bennett, Jr., L. L. *The Synthesis of Carbocyclic Analogs of Purine Nucleosides Related to Nucleoside (1)*. *J. Am. Chem. Soc.* 1971, 93, 4320-4324.
- (23) Schreier, S.; Allen, P. W.; Bennett, Jr., L. L. *The Synthesis of Carbocyclic Analogs of Purine Nucleosides Related to Nucleoside (1)*. *J. Am. Chem. Soc.* 1972, 94, 3346-3357.
- (24) Schaeffer, H. J.; Gierke, S.; Hines, R.; Hittner, S.; Vogel, S.; Schaeffer, H. J. *Anticancer Res.* 1981, 1, 307-308.

Scheme 1



* (a) NaH, DMF; (b) H₂SO₄/Cl₂/DMF; (c) (n-Bu)₃Sn₂O; benzene; (d) H₂SO₄/Cl₂/DMF; (e) CF₃CO₂H, H₂O.

Scheme II



* (a) acetone, p-TsOH, 2,2-dimethoxypropane; (b) 40% NH₄OH in H₂O, 85 °C; (c) 40% NH₄OH, 100 °C; (d) NaH, DMF; (e) H₂SO₄/Cl₂/DMF; (f) CF₃CO₂H, H₂O.

(9), first reported by Shady et al.,²⁵ was reacted under these conditions, intermediate 10 was isolated in a 82% yield (Scheme II). When 9 was activated with bis(tributyltin)oxide and then reacted with sulfamoyl chloride, 10 was obtained in a 34% yield. Because of the better yield obtained with sodium hydride, this method was used for all further sulfamoylations. Compound 10 was easily deprotected in aqueous trifluoroacetic acid to give the first target molecule 2.

The 6-chloropurinyl carbocyclic nucleoside²⁶ 11, was protected as the acetonide to give 12 (Scheme III). The 6-chloro group in 12 was easily displaced with methylamine

- (25) Shady, Y. F.; Clayton, J. D. *Synthesis of Carbocyclic Analogs of Purine Ribonucleosides*. *J. Am. Chem. Soc.* 1983, 105, 3075-3083.
- (26) Shady, Y. F.; Clayton, J. D. *Carbocyclic Analogs of 6-Substituted Purine Ribonucleosides and of Adenosine Ribonucleosides*. *J. Pharm. Sci.* 1974, 63, 1121-1127.

Table I. Cytotoxicity to P388 Mouse Leukemia Cells

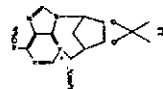
	X	Z	Y	IC ₅₀ (μg/mL)
1	NH ₂	CH	OSO ₂ NH ₂	0.002-0.126
2	NHCH ₃	CH	OSO ₂ NH ₂	2-4
3	NHCH ₃	CH	OSO ₂ NH ₂	~74
4	NHCH ₃	CH	OSO ₂ NH ₂	~50
5	SC(=O)H	N	OSO ₂ NH ₂	0.0166-0.0925
6	SC(=O)H	N	OSO ₂ NH ₂	>100
7	SC(=O)H	N	OSO ₂ NH ₂	>100
8 (acyclo)	NH ₂	CH	OH	~0.6
9	NH ₂	CH	OH	0.25-0.5
10	NH ₂	CH	OH	0.25-0.5
11	NH ₂	CH	OH	0.25-0.5
12	NH ₂	CH	OH	0.25-0.5
13	NH ₂	CH	OH	0.25-0.5
14	NH ₂	CH	OH	0.25-0.5
15	NH ₂	CH	OH	0.25-0.5
16	NH ₂	CH	OH	0.25-0.5
17	NH ₂	CH	OH	0.25-0.5
18	NH ₂	CH	OH	0.25-0.5
19	NH ₂	CH	OH	0.25-0.5
20	NH ₂	CH	OH	0.25-0.5
21	NH ₂	CH	OH	0.25-0.5
22	NH ₂	CH	OH	0.25-0.5
23	NH ₂	CH	OH	0.25-0.5
24	NH ₂	CH	OH	0.25-0.5
25	NH ₂	CH	OH	0.25-0.5
26	NH ₂	CH	OH	0.25-0.5
27	NH ₂	CH	OH	0.25-0.5
28	NH ₂	CH	OH	0.25-0.5
29	NH ₂	CH	OH	0.25-0.5
30	NH ₂	CH	OH	0.25-0.5
31	NH ₂	CH	OH	0.25-0.5
32	NH ₂	CH	OH	0.25-0.5
33	NH ₂	CH	OH	0.25-0.5
34	NH ₂	CH	OH	0.25-0.5
35	NH ₂	CH	OH	0.25-0.5
36	NH ₂	CH	OH	0.25-0.5
37	NH ₂	CH	OH	0.25-0.5
38	NH ₂	CH	OH	0.25-0.5
39	NH ₂	CH	OH	0.25-0.5
40	NH ₂	CH	OH	0.25-0.5
41	NH ₂	CH	OH	0.25-0.5
42	NH ₂	CH	OH	0.25-0.5
43	NH ₂	CH	OH	0.25-0.5
44	NH ₂	CH	OH	0.25-0.5
45	NH ₂	CH	OH	0.25-0.5
46	NH ₂	CH	OH	0.25-0.5
47	NH ₂	CH	OH	0.25-0.5
48	NH ₂	CH	OH	0.25-0.5
49	NH ₂	CH	OH	0.25-0.5
50	NH ₂	CH	OH	0.25-0.5
51	NH ₂	CH	OH	0.25-0.5
52	NH ₂	CH	OH	0.25-0.5
53	NH ₂	CH	OH	0.25-0.5
54	NH ₂	CH	OH	0.25-0.5
55	NH ₂	CH	OH	0.25-0.5
56	NH ₂	CH	OH	0.25-0.5
57	NH ₂	CH	OH	0.25-0.5
58	NH ₂	CH	OH	0.25-0.5
59	NH ₂	CH	OH	0.25-0.5
60	NH ₂	CH	OH	0.25-0.5
61	NH ₂	CH	OH	0.25-0.5
62	NH ₂	CH	OH	0.25-0.5
63	NH ₂	CH	OH	0.25-0.5
64	NH ₂	CH	OH	0.25-0.5
65	NH ₂	CH	OH	0.25-0.5
66	NH ₂	CH	OH	0.25-0.5
67	NH ₂	CH	OH	0.25-0.5
68	NH ₂	CH	OH	0.25-0.5
69	NH ₂	CH	OH	0.25-0.5
70	NH ₂	CH	OH	0.25-0.5
71	NH ₂	CH	OH	0.25-0.5
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73	NH ₂	CH	OH	0.25-0.5
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76	NH ₂	CH	OH	0.25-0.5
77	NH ₂	CH	OH	0.25-0.5
78	NH ₂	CH	OH	0.25-0.5
79	NH ₂	CH	OH	0.25-0.5
80	NH ₂	CH	OH	0.25-0.5
81	NH ₂	CH	OH	0.25-0.5
82	NH ₂	CH	OH	0.25-0.5
83	NH ₂	CH	OH	0.25-0.5
84	NH ₂	CH	OH	0.25-0.5
85	NH ₂	CH	OH	0.25-0.5
86	NH ₂	CH	OH	0.25-0.5
87	NH ₂	CH	OH	0.25-0.5
88	NH ₂	CH	OH	0.25-0.5
89	NH ₂	CH	OH	0.25-0.5
90	NH ₂	CH	OH	0.25-0.5
91	NH ₂	CH	OH	0.25-0.5
92	NH ₂	CH	OH	0.25-0.5
93	NH ₂	CH	OH	0.25-0.5
94	NH ₂	CH	OH	0.25-0.5
95	NH ₂	CH	OH	0.25-0.5
96	NH ₂	CH	OH	0.25-0.5
97	NH ₂	CH	OH	0.25-0.5
98	NH ₂	CH	OH	0.25-0.5
99	NH ₂	CH	OH	0.25-0.5
100	NH ₂	CH	OH	0.25-0.5



and dimethylamine to produce intermediates 13 and 14, respectively. The 6-(thiomethyl) derivative was approached by first forming the 6-thiopurine from 8-chloropurine intermediate 12, followed by methylation. However, in one experiment, displacement of the chlorine with a thiol using thiourea in refluxing propanol effected not only the formation of the 6-thiopurine, but also the deprotection of the acetone. A potential cause for this deprotection could be trace amounts of acid and water in the propanol used. Because of this unusual result, the 6-(thiomethyl) nucleoside 15 was synthesized from 11 as described by Shealy.²⁹ The diol in 15 was protected with an acetone to give 16. 8-Aza carbocyclic adenine (17), was similarly protected to give acetone 18.

Treatment of methylamine (19), methylthio (16), and 8-azadenosine (18) derivatives with sodium hydride and sulfamoyl chloride furnished the sulfamoylated protected intermediates 19-21, respectively. Deprotection of the diols in aqueous trifluoroacetic acid provided the three target molecules 3, 5, and 6, respectively (Scheme III).

The dimethylamine derivative 14 was also reacted with sodium hydride and sulfamoyl chloride; however, a pure sample of the sulfamoylated intermediate 22 was never isolated (Scheme III). The proton NMR spectrum of the impure product indicated that some anhydro nucleoside (23) may have formed via N3,C5'-cyclization. Normally, H2 and H8 of the purine ring in N3,N'-dimethyl bases occur at ~8.28 and ~8.21 ppm in dimethyl sulfoxide (as in compound 14). However, when an anhydro derivative forms, these protons are shifted downfield. For example, in adenine the shift occurs from 7.9 and 8.25 to 8.66 and 8.78.³⁰ In the impure sample of 22, peaks were seen at 8.67 and 8.48 ppm in addition to the normal purine peaks at 8.28 and 8.21, suggesting an anhydro impurity. Triethylamine was therefore explored as a 6'-hydroxyl activating agent to see if this weaker base could somehow offset the N3 to C5'-cyclization. However, the intermediate 22 produced using triethylamine contained many impurities. Deprotection of the impure sample yielded target molecule 4 in a 33% yield from 14. Both sodium hydride and triethylamine conditions led to compound 4; however, the best yield was obtained when triethylamine was used in the reaction sequence.



Biological Results and Discussion

The compounds in Table I were evaluated for cytotoxicity against P388 mouse leukemia cells.³¹ Puromycin, a

(29) (a) Shealy, Y. F.; Clayton, J. D. Carbocyclic Analogs of Purine Nucleosides with Antileukemic Activity. *J. Pharm. Sci.* 1973, 62, 893-900. (b) Shealy, Y. F.; Clayton, J. D. Carbocyclic Analogs of 8-Azadenosine and 8-Azadenine. Carbocyclic Analogs of 8-Azadenosine and 8-Azadenine. *J. Heterocycl. Chem.* 1973, 10, 901-906. (30) Thompson, L. B. Nuclear Magnetic Resonance Spectroscopy in the Study of Purine and Pyrimidine Nucleosides. In *Synthetic Procedures in Nucleic Acid Chemistry*; Zareba, W. N., Tyson, R. S., Eds.; John Wiley and Sons: New York, 1973; Vol. 2, pp 257-306.

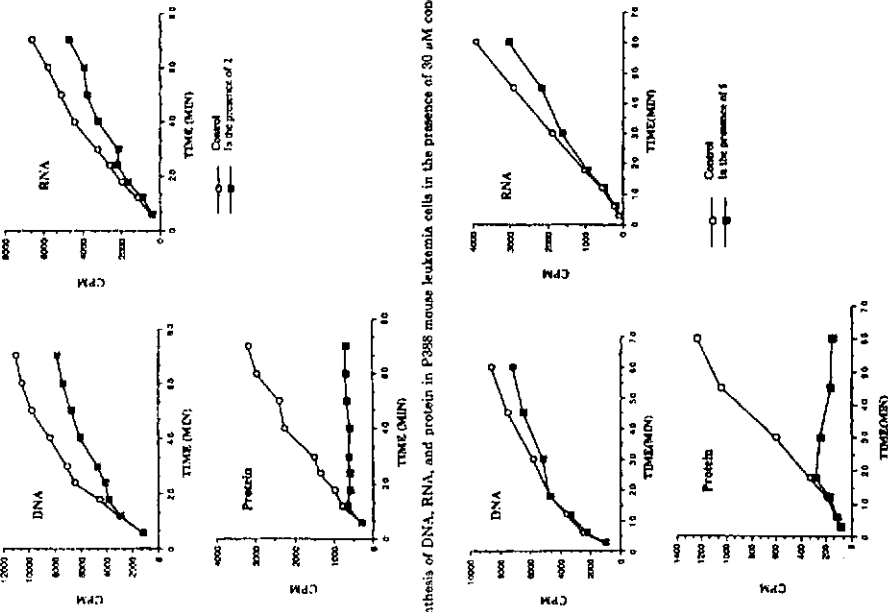


Figure 1. Bioassays of DNA, RNA, and protein in P388 mouse leukemia cells in the presence of 30 μM concentration of agent 2.

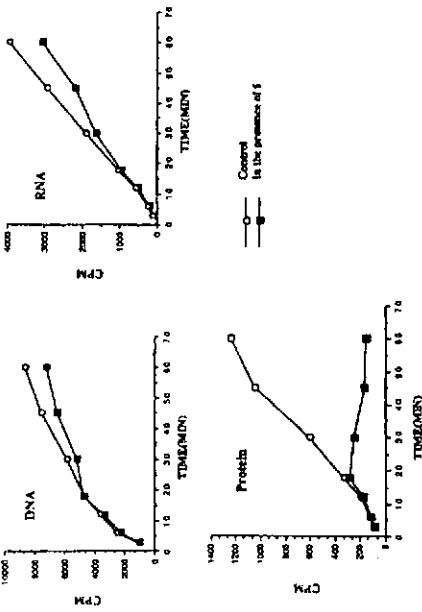


Figure 2. Bioassays of DNA, RNA, and protein in P388 mouse leukemia cells in the presence of 12 μM concentration of agent 5.

study, represented in Figure 4, however, indicated that neither compound 2 nor compound 5 inhibited protein biosynthesis to any great extent except at very high concentrations. At concentrations of 1×10^{-4} M, agents 2 and 5 inhibited polyphenylalanine synthesis by approximately 40%. On the other hand, puromycin, an agent known to act directly on protein biosynthesis, inhibited the synthesis by 89% at a concentration of 3×10^{-4} M. Thus, this study suggested that (1) protein biosynthesis may not be directly affected by agents 2 and 5 or (2) these agents were selective for eukaryotic systems.

Since protein biosynthesis in the *E. coli* ribosomal system appeared to be unaffected by agents 2 and 5, a prokaryotic whole cell system, *E. coli* bacteria, was therefore investigated. The *E. coli* was incubated in the presence of each

test agent, and bacterial growth was measured by optical density. Chloramphenicol, a prokaryotic-specific protein biosynthesis inhibitor, was used as a positive control. The results of this assay are shown in Figure 5. None of the new agents (2-5, 8) exhibited significant antibacterial activity except at very high concentrations. These results support the hypothesis that agents 2 and 5 are selectively toxic to eukaryotic systems.

In order to further confirm that agents 2 and 5 were acting on eukaryotic protein biosynthesis, a rabbit reticulocyte lysate system was explored. By incubating this system with a radioactive amino acid and agent 2 or agent 5, the effectiveness of these agents at inhibiting eukaryotic protein biosynthesis was determined. The target molecules (2 and 5) were thus incubated with the reticulocyte

SS4

2-methylcyclopentyl)-1- α -(methoxymethyl)cyclopentyl]-8-methoxythio)purine (16, 0.471 g, 1.4 mmol), NaH (~0.200 g, 8.3

The crystals were identified as impure sulfamoyl chloride ($\text{NH}_2\text{SO}_2\text{Cl}$) and were stored in the freezer for use in future reactions.

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(A, 2 H, H_2NSO_3), 7.18 (s, 2 H, C₆NH₂), 5.07 (d, J = 8.4, 1 H, C²OH), 4.89 (d, J = 4.4, 1 H, C³OH), 4.69 (m, 1 H, C⁴H), 4.38

N-[6-(methoxymethyl)purine-2 α], ($\pm$)-9-[2-methoxy-4 β -(hydroxymethyl)cyclopentyl]-6- α -D-glucopyranoside. Yield, 0.471 g, 1.4 mmol; NMR ($\sim$ 0.200 g, 3.3

and weighed 3.49 g (80%, impure).
(2a, 3a-Di-*O*-isopropyl
methylcyclopentyl
(methylthio)purine (

The crystals were identical to those obtained from the SO_2Cl_2 and were stored under nitrogen.

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thorance at 485 nm was followed for up to 10 min. A decrease in absorbance indicated that the reaction was occurring and thus, that a compound is a substrate for this enzyme. Agent 2 showed only a slight decrease in absorbance after 10 min and was therefore considered a slow substrate.

Adenylic Acid Deaminase Assay. In the standard assay procedure, 1 mL of citric acid buffer (0.01 M, pH 8.3) and rabbit muscle adenylic acid deaminase (50 millunits) were combined with AMP or 2. (Note: One unit of enzyme will convert one micromole of 5'-AMP to 5'-IMP per minute at 25 °C at pH 8.5). The enzymatic reaction was run at 25 °C and absorbance at 265 nm was followed. A decrease in absorbance indicated that the reaction was occurring and, thus, that a compound is a substrate for this enzyme. No decrease in absorbance was seen when agent 2 was incubated with the enzyme; therefore, this agent was not a substrate.

Acknowledgment. We gratefully acknowledge the support of this work by the National Cancer Institute, Grant CA23263.

solution was provided which was devoid of leucine. The standard assay procedure described in the kit was performed. Tobacco mosaic virus RNA was used as the messenger RNA at a final concentration of 1 µg/mL. [¹⁴C]-leucine was used as the amino acid label. At the end of the 90-min incubation at 30 °C, the reaction mixture was terminated with sodium hydroxide and combined with 10% TCA (2 mL) to precipitate the protein. The mixture was filtered through a Millipore type HA filter (0.45 µm) and washed with ice-cold 5% TCA (4 × 3 mL). The filters were described above. Various concentrations of agents 2 and 6 and cyclohexamide were incubated with the reticulocyte system. The percent inhibition of protein biosynthesis in the presence of these agents was determined and is depicted in Figure 6.

Adenosine Deaminase Assay. In the standard assay procedure, 1 mL of potassium phosphate buffer (0.05 M, pH 7.4) and calf intestinal mucosa adenosine deaminase (20 millunits) were combined with adenosine or 2. (Note: One unit of enzyme will deaminate one micromole of adenosine per minute at 25 °C at pH 7.5). The enzymatic reaction was run at 25 °C and the

centrifuged for 5 min at 2700 to pellet the cells. The supernatant was then decanted and the remaining cell pellet was resuspended in 2 mL of RBSS and centrifuged as before. The supernatant was again decanted and the pellet resuspended in 1 mL of water to lyse the cells. The water mixture was placed in an ice bath for 1 h, and 2 mL of cold 10% trichloroacetic acid (TCA) was added to precipitate the macromolecules. The acidic mixture was kept in an ice bath for 15 min, and then the mixture was filtered through a Millipore type HA filter (0.45 µm). The filter was washed with 3 × 2 mL of ice-cold 5% TCA, and then the filter was dried under a heat lamp for 30 min. The dry filter was suspended in 10 mL of Ecolume (ICN), and radioactivity was counted via liquid scintillation. The number of counts reflects the amount of radioactive amino acid incorporation into precipitated protein. Thus, the number of counts is proportional to the amount of protein synthesis taking place.

A very similar assay was used for the RNA and DNA studies. The only difference was that the [¹⁴C]-amino acids were replaced with [¹⁴C]-2-uridine for the RNA assay and [¹⁴C]-thymidine for the DNA assay. The results of the studies are depicted in Figures 1, 2, and 3.

Poly(cytidylic-Uridylic Acid)-Directed Synthesis of Polyphenylalanine. The *E. coli* protein biosynthesis assays were essentially as described previously.^{22a} Compounds 2 and 6 and puramycin were dissolved in water containing 10% DMSO. In a series of test tubes chilled on ice, solutions of one agent (test) or water (control) were combined with cell-free protein biosynthesis components devoid of the messenger RNA and also containing [¹⁴C]-Phe. Various concentrations of the test compounds were prepared. Protein biosynthesis was initiated by adding poly(cytidylic-uridylic acid) to each tube and then incubating the tubes at 37 °C (water bath) for 30 min. The protein synthesis was stopped by adding sodium hydroxide to the tubes and incubating for an additional 15 min at 37 °C. Hydrochloric acid was then added to neutralize the mixture. The tubes were placed in an ice bath and combined with 10% TCA (2 mL per tube) to precipitate the proteins. The contents of the tubes were then filtered through Millipore type HA filters (0.45 µm pore size) and washed with ice-cold 5% TCA (3 × 2 mL). The filters were dried under a heat lamp for 30 min, combined with 10 mL of Ecolume in scintillation vials, and counted with a liquid scintillation counter. Radioactive counts reflect [¹⁴C]-Phe incorporation into protein; thus, the number of counts is proportional to the amount of protein biosynthesis occurring. The percent inhibition of protein biosynthesis was determined for 2, 6, and puramycin at various concentrations by comparing the number of counts in the test and control vials. The results of this study was presented in Figure 4.

Antibacterial Assay. *E. coli* B210 bacteria were grown overnight in nutrient broth media at 37 °C in a shaking water bath. (Nutrient broth media contains 9 g of nutrient broth and 10 g of dextrose in a 1-L aqueous solution.) The compounds used for testing for antibacterial action and chloramphenicol, the positive control, were prepared as aqueous solutions containing 2% DMSO. These solutions were sterilized by passage through Millipore type HA filters (0.22-µm pore size). 3-fold serial dilutions of the agents in 1.5 mL of water were prepared in sterile tubes equipped with sterile cotton plugs. An inoculant of an overnight culture of bacterial was suspended in 2 × nutrient broth media, and 1.5 mL of this suspension was added to each tube in the series. The 3-mL mixtures in the tubes were incubated at 32 °C in a water bath. Bacterial cell growth was followed by increase in optical density at 485 nm using a Spectronic 20 spectrophotometer. The tubes were monitored for up to 24 h, and the results are presented in Figure 5.

Rabbit Reticulocyte Lysate Assay. A commercially available translation kit was purchased from Boehringer Mannheim. The kit contained nuclease-treated rabbit reticulocyte lysate which was depleted to amino acids; however, an amino acid

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each 1 H, C2 and C8H₄, 1.52 (s, 2 H, H₂NSO₂), 1.48, 1.23 (each s, each 3 H, CCH₃), which are characteristic of intermediate 21. Also, MS (FAB) shows a signal at 411 (M⁺-H)⁺. The material was dissolved in 90% aqueous trifluoroacetic acid (15 mL), and the solution was stirred at room temperature for 2 h. The solution was reduced in volume to a white residue and this was dissolved in methanolic ammonia (1:1, 10 mL). The solvent was evaporated from the basic solution, and a clear syrup remained. Crystallization of this syrup from water produced a white solid which was identified as 4: total yield from 14, 0.085 g, 33%; mp 124-126 °C; *R*_f = 0.36 (concentrated ammonia-propagol, 1:4); UV: ϵ_{254} 269, 213 (0.1 N HCl); 277, 213 (H₂O); 276, 219 (0.1 N NaOH); IR (cm⁻¹) (3.77, 3114) (broad, O-H), (1608, 1565) (C=O, C=N), 1321, 1134, 1033 (S=O); ¹H NMR (Me₂SO-*d*₆) δ 8.19 (s, 2 H, C2 and C8H₄), 7.49 (s, 2 H, H₂NSO₂), 5.05 (d, *J* = 6.5, 1 H, C2'H), 4.89 (d, *J* = 4.5, 1 H, C3'OH), 4.72 (m, 1 H, C1'H), 4.38 (m, 1 H, C2'H), 4.18, 4.07 (each m, each 1 H, C5'H₂), 3.87 (m, 1 H, C3'H), 3.45 (s, 6 H, A₂N(CH₃)₂), 2.32 (m, 2 H, 1,74 (m, 1 H) (C6'H₃ and C4'H); signals at 7.49, 5.05, and 4.89 are D₂O exchangeable; MS (FAB, thioglycerol) 373 (M⁺ + H)⁺. Anal. (C₁₂H₁₂N₄O₈S) C, H, N.

Biology. The Fischer media, horse serum, and Earl's balanced salt solution were purchased from Gibco, Inc. An RPMI 1640 amino acid kit was used to prepare the modified media. The [¹⁴C]-2-uridine (68.8 mCi/mmol), [¹⁴C]-L-leucine (300 mCi/mmol), and [¹⁴C]-L-lysine (306 mCi/mmol) were obtained from New England Nuclear, Inc. The [¹⁴C]-thymidine (61.4 mCi/mmol) was purchased from Amersham, and the [¹⁴C]-L-phenylalanine (405 mCi/mmol) was purchased from ICN. Centrifugation was done on a Sorvall RC2B instrument. Liquid scintillation was purchased using a Beckman LS 6800 counter. HA type filters were purchased from Millipore Corp. Pentane liquid scintillation fluid was purchased from ICN. The *E. coli* paste used to prepare the ribosomes was purchased from Grain Processing Corp., Muscatine, IA. *E. coli* B210 was obtained from the U.S. Dept. of Agriculture. The bacterial nutrient broth was purchased from Difco. The optical densities in the bacterial study were determined on a Bausch and Lomb (Spectronic 20) spectrophotometer. Buffer A contains 0.05 M potassium chloride, 0.01 M magnesium acetate, and 0.01 M Tris acetate (pH 7.5). Adenosine deaminase and adenylic acid deaminase were purchased from Sigma, Inc. A Beckman Model 25 UV spectrophotometer was used to evaluate the deaminase results.

Antitumor Assay. The compounds in Table I were evaluated as in vitro antitumor agents by using the protocol of the National Cancer Institute.²⁷ The methodology used was described in ref. 31. P388 Mouse Leukemia cells grown in Fiebers media supplemented with 10% horse serum were incubated with and without (control) the compounds under study at various concentrations. The cells had a starting concentration of ~10⁶ cells/mL and were incubated at 37 °C for 3 days. Cell growth was determined with a hemacytometer, and puramycin was used as a positive control. The approximate concentration of each test compound which inhibits cell growth by 50% (IC₅₀) is reported in Table I.

Assay for DNA, RNA, and Protein Biosynthesis. A modified Fischer media was prepared which contained only half the recommended concentrations of L-phenylalanine (25, 30 mg/L used), L-leucine (10, 15 mg/L used), and L-lysine (10, 20 mg/L used). For the protein assay, 10 µL each of [¹⁴C]-Phe, [¹⁴C]-Leu, and [¹⁴C]-Lys were added to 1 mL of the modified media. One microliter of this radioactive solution was then removed for background counts. The remaining 10 mL was separated into two 5-mL portions. Ten microliters of the sample were added to each portion. Ten microliters of the control solution were added to each portion. The tubes were then combined with 5 mL of P388 cells suspended in the modified media to give a final cell concentration of 10⁶ cells/mL. The 10-mL portions were incubated at 37 °C (water bath) and every 9-10 min a 1-mL fraction was removed from each portion and added to 1 mL of chilled (ice bath) Earl's balanced salt solution (RBSS). Each fraction was then treated as follows. The RBSS solution was

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