

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



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Señor

SUPERINTENDENTE DE INDUSTRIA Y COMERCIO

DIVISION DE NUEVAS CREACIONES

E. S. D.

Re: P1.-PHARMACIA ITALIA S.p.A.-Solicitud de Patente de Invención en Fase Nacional PCT para "COMPOSICIONES FARMACÉUTICAS QUE COMPRENDEN DERIVADOS DE ACRILOIL DISTAMICINA E INHIBIDORES DE TOPOISOMERASA I Y II".-Clase A61K 31/70.-Expediente número 03-003.253.-

1. Me refiero al auto dictado por ese Despacho notificado por fijación en lista de Abril 4 de 2008.

2. Con el fin de responder las objeciones presentadas por el Señor Examinador en su Concepto Técnico **No. 928** bajo los acápites de claridad de la solicitud, reivindicaciones relativas a uso y novedad, me permito dirigir su atención a las siguientes modificaciones y argumentos técnicos, por medio de los cuales se superan completamente dichas objeciones.

3. **CLARIDAD DE LA SOLICITUD.**

3.1. **Descripción.**

En primera instancia el Señor Examinador señala que la descripción de la presente solicitud no brinda sustento suficiente para reclamar todas las posibles combinaciones reivindicadas, porque sólo se revelan resultados experimentales de un compuesto preferido de la invención combinado con doxorubicina.

Sobre este particular me permito manifestar que, tal como se discutirá detalladamente en este mismo memorial, el capítulo reivindicatorio fue modificado de forma tal que se especifican claramente el derivado de distamicina y los inhibidores de topoisomerasa que hacen parte de los productos de la invención.

Consecuentemente, el capítulo descriptivo, tal como está presentado, brinda total sustento a las nuevas reivindicaciones que reclaman productos que combinan el derivado de distamicina, el PNU 166196, y doxorubicina ó CPT-11.

Tal como lo muestra la tabla 1 de la descripción de la solicitud en estudio (páginas 12 y 13), esta combinación del compuesto preferido de la invención con doxorubicina sorprendentemente brinda un aumento significativo en el ILS% de ratas ($ILS\% = [(tiempo\ medio\ de\ supervivencia\ de\ ratas\ tratadas / tiempo\ medio\ de\ supervivencia\ de\ ratas\ control) \times 100] - 100$), sin producir toxicidad, siendo este efecto más que aditivo, y por lo tanto sinérgico.

Me permito señalar que dicho efecto sinérgico sorprendente que se obtiene de la combinación del derivado de distamicina PNU 166196 y doxorubicina ó CPT-11, es igualmente comprobado con los resultados consignados en el documento *Clin. Cancer Res.*, **2003**,9, 5402-5408, de publicación posterior a la presente solicitud.

En la tabla 2 del citado documento (página 5406), se resumen los resultados obtenidos al combinar el compuesto preferido de la invención (PNU 166196, denominado en el artículo brostalicina) con CPT-11, en el tratamiento de carcinomas de colon humano HCT-116 injertados en ratas.

En dicha tabla es evidente que el empleo de CPT-11 con brostalicina, por ejemplo en dosis de 60 y 0.4 mg/Kg/inyección respectivamente, aumenta significativamente la regresión del tumor (T/C%), siendo dicha regresión mucho mayor que cuando se emplean cada uno de los principios activos por separado ($T/C\% = 100 \times (RTV\ medio\ del\ grupo\ tratado) / (RTV\ medio\ del\ grupo\ control)$, $RTV = TV_n / TV_0$, TV_n = volumen del tumor en el día n, TV_0 = volumen del tumor en el día 0).

Dose (mg/kg/injection)		T/C (%) ^a	FTV ^b	LCK ^c	T-C (days) ^d	Toxic death
CPT-11	Brostallicin					
0	0.2	80	—	0.1	2	0/8
0	0.4	68	—	0.4	11	0/8
40	0	46	—	0.4	11	0/8
60	0	39	—	0.8	23	0/8
40	0.2	28 ^{e,f}	1.8	1.1	33	0/8
40	0.4	32 ^{e,f}	1.1	0.9	26	0/8
60	0.2	29 ^f	1.4	1	31	0/8
60	0.4	19 ^{e,f}	1.2	1.7	51	0/8

^a Tumor regression (T/C%) on day 7 after the last treatment.

^b Fractional tumor volume relative to controls was calculated as described in "Materials and Methods."

^c Log cell kill.

^d T-C, tumor growth delay.

^e $P < 0.05$ versus CPT-11 alone.

^f $P < 0.01$ versus brostallicin alone.

De igual forma, la fracción de volumen relativa del tumor (FTV) demuestra que al usar conjuntamente la brostalicina y el CPT-11, el efecto obtenido sobre el tumor es más que aditivo (FTV= relación entre el volumen medio del tumor del grupo tratado con respecto al volumen medio del tumor del grupo control, una relación >1 indica un efecto más que aditivo), por ejemplo, a dosis de 60 mg/Kg/inyección de CPT-11 y 0.4 mg/Kg/inyección de brostalicina el valor del FTV es de 1.2.

Este hecho es igualmente comprobado por los resultados de retraso de crecimiento del tumor (T-C) obtenidos con la máxima dosis de los dos principios activos. Estos resultados muestran como el tumor de los animales tratados con la combinación de los dos principios activos retrasa significativamente su crecimiento con respecto al tumor de los animales control, con un T-C de 51, que difiere significativamente del obtenido con el tratamiento de CPT-11 (máximo de 11) ó PNU 166196 por separado (máximo de 23). (T-C= tiempo medio en días requerido para que el tumor del grupo tratado llegue a un tamaño determinado (generalmente 1g), menos el tiempo medio para que el tumor del grupo control llegue al mismo tamaño).

De esta forma, el solicitante comprobó que la combinación de PNU 166196 y CPT-11, sorprendentemente brinda un mejor comportamiento antitumoral que cada uno de los compuestos solo.

Un comportamiento similar fue obtenido de la combinación de PNU 166196 con doxorubicina, tal como lo resume la tabla 3 del mismo documento:

Dose (mg/kg/day)		Schedule 1 DX (day 1) Brostallicin (day 1)			Schedule 2 DX (day 1) Brostallicin (day 2)			Schedule 3 Brostallicin (day 1) DX (day 2)				
		Brostallicin	DX	ILS% ^a	Tox ^b	CI ^c	ILS% ^a	Tox ^b	CI ^c	ILS% ^a	Tox ^b	CI ^c
0.2	0			38	0/8	—	8	0/8	—	14	0/8	—
0.4	0			23	0/8	—	17	0/8	—	14	0/8	—
0.8	0			69	0/8	—	33	0/8	—	50	0/8	—
0	7.5			54	0/8	—	33	0/8	—	29	0/8	—
0	15			100	0/8	—	67	0/8	—	57	0/8	—
0.2	7.5			69	0/8	0.75	42	0/8	1.02	57	0/8	1.33
0.2	15			92	0/8	0.67	100	0/8	1.33	86	0/8	1.21
0.4	7.5			78	0/8	1.01	58	0/8	1.16	71	0/8	<u>1.65</u>
0.4	15			108	0/6	0.88	100	0/8	1.19	93	0/8	1.31
0.8	7.5			100	0/8	0.81	100	0/8	<u>1.52</u>	86	0/8	1.1
0.8	15			123	0/8	0.73	100	0/8	1	<u>114</u>	0/8	1.1

^a Percentage increase in life span.
^b Number of toxic deaths/number of mice.
^c Combination index = ratio between the ILS% of the two drugs in combination and the sum of ILS% of brostallicin and DX alone. A CI of >1 indicates a more than additive effect, and a ratio of <1 indicates a less than additive effect.

En dicha tabla se muestra que la combinación del PNU 166196 con doxorubicina puede aumentar significativamente el ILS%, llegando hasta valores de 114. Adicionalmente, el índice de combinación calculado para estos tratamientos (IC) comprueba que el efecto producido es más que aditivo (IC= relación entre ILS% de los dos medicamentos en combinación y la suma de ILS% de brostalicina y doxorubicina solas, una relación >1 indica un efecto más que aditivo), con valores de por ejemplo 1.52 y 1.65.

Estos resultados se constituyen en una prueba del efecto sinérgico sorprendente que se obtiene al emplear conjuntamente el compuesto preferido de la invención, PNU 166196, combinado con un inhibidor de topoisomerasa como es el CPT-11 o la doxorubicina.

De esta forma, la descripción de la solicitud en estudio muestra de forma explícita el efecto técnico sorprendente que presenta la combinación que ahora es reclamada dentro del capítulo reivindicatorio, y, por lo tanto, dicha descripción es clara y suficiente al revelar los productos de la invención.

3.2. **Reivindicaciones.**

Por otro lado, el Señor Examinador objeta la claridad del capítulo reivindicatorio por considerar que no se identifican de forma inequívoca los compuestos que conforman los productos de la invención, sino se hace referencia a grupos farmacofóricos o categorías farmacológicas, y de esta forma no es posible precisar el alcance de la invención.

Además resalta que el capítulo reivindicatorio carece de concisión, porque, según su concepto, repite innecesariamente las características técnicas de la invención en dos reivindicaciones independientes.

Acatando estas objeciones es deseo del solicitante presentar un **nuevo capítulo reivindicatorio, que reemplaza el anterior, constituido por 3 reivindicaciones**, en las cuales se definen completamente el derivado de distamicina y los inhibidores de topoisomerasa que son parte de los productos de la invención.

Me permito señalar que este nuevo capítulo reivindicatorio no amplía de forma alguna el alcance de la invención, como fue originalmente presentada, toda vez que los compuestos a los cuales se hace referencia están explícitamente revelados en la descripción como me permito mostrar a continuación:

- Nueva Reivindicación 1. La nueva reivindicación 1 hace referencia a los productos de la invención que contienen específicamente un derivado de distamicina preferido de la invención, el PNU166196, revelado en la descripción de la presente solicitud (páginas 6 y 12), junto con un inhibidor de topoisomerasa seleccionado entre doxorubicina y CPT-11, tal como se considera en la página 9 de la descripción.

De esta forma, la nueva reivindicación 1 se lee como sigue:

"Productos que comprenden clorhidrato de N-(5-{{(5-{{(5-{{(2-{{amino(imino)metil}amino} etil) amino]carbonil}-1-metil-1H-pirrol-3-il)amino]carbonil}-1-metil-1H-pirrol-3-il)amino]carbonil}-1-metil-1H-pirrol-3-il)-4-[(2-bromoacrilil)amino]-1-metil-1H-pirrol-2-carboxamida y un inhibidor de topoisomerasa seleccionado del grupo que consiste de doxorubicina y CPT-11 como una preparación combinada para uso simultáneo, separado o secuencial en el tratamiento de tumores."

- Nueva Reivindicación 2. Hace referencia al caso particular en el cual en el producto de la invención el inhibidor de topoisomerasa es el CPT-11.
- Nueva Reivindicación 3. Considera el caso particular en el que el inhibidor de topoisomerasa presente en el producto de la invención es la doxorubicina

De esta forma, el nuevo capítulo reivindicatorio es completamente claro al reclamar el producto materia de la presente solicitud, definiendo de inequívocamente los principios activos presentes en dicho producto de la invención. Así mismo, las nuevas reivindicaciones evitan la repetición innecesaria de cláusulas que comprendan características técnicas iguales.

Consecuentemente, me permito resaltar que el nuevo capítulo reivindicatorio es claro y conciso al reclamar el producto objeto de la presente invención,

cumpliendo por lo tanto con lo preceptuado en el Artículo 30 de la Decisión 486 de 2000.

4. **REIVINDICACIONES RELATIVAS A USO.**

De otro lado, el Señor Examinador señala que las reivindicaciones 13 a 19 presentadas se refieren a usos y no a productos ó procedimientos, siendo por tanto no patentables, como lo considera el Artículo 14 de la Decisión 486 de 2000.

Me permito señalar que, tal como se expuso anteriormente, **el nuevo capítulo reivindicatorio no incluye reivindicaciones relativas a usos**, por lo que dicho capítulo reivindicatorio se ajusta completamente a los lineamientos establecidos por la Decisión 486 de 2000.

5. **NOVEDAD.**

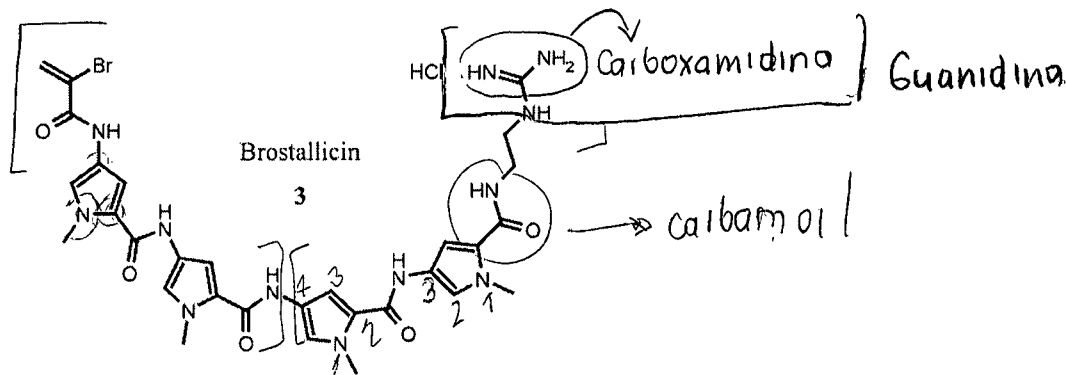
Finalmente, el Señor Examinador objeta la novedad de la solicitud en estudio a la luz de las enseñanzas de los documentos **WO9804524 (D1)**, **WO9950266 (D2)** y **WO9821202 (D3)**.

Con respecto a estos documentos el Señor Examinador asegura que "*según las anterioridades D1, D2 ó D3 ya era posible combinar un derivado de acrilolil amida conforme a la fórmula (I) con un inhibidor de topoisomerasa con el animo de alcanzar un efecto terapéutico superior frente al tratamiento convencional del cáncer*".

A este respecto me permito manifestar que, si bien es cierto que los documentos citados hacen referencia a derivados de distamicina, dichos documentos fueron referenciados por el solicitante dentro de la descripción de la solicitud en estudio como arte previo (página 1 de la descripción), y no contienen todas las características técnicas de la invención, por lo que no anticipan de forma alguna los productos revelados en la solicitud en estudio.

Las diferencias existentes entre dichos documentos y la presente solicitud son más claras a la luz de las modificaciones realizadas sobre el capítulo reivindicatorio, anteriormente discutidas.

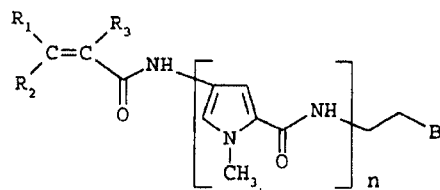
Dichas modificaciones, establecen que el derivado preferido de la invención corresponde al clorhidrato de N-(5-[[[(5-[[[(5-[[[(2-[[amino(imino)metil]amino]etil) amino]carbonil}-1-metil-1H-pirrol-3-il)amino]carbonil}-1-metil-1H-pirrol-3-il)amino]carbonil}-1-metil-1H-pirrol-3-il)-4-[(2-bromoacrilil)amino]-1-metil-1H-pirrol-2-carboxamida, PNU 166196, o brostalicina, cuya estructura es la siguiente:



Ninguno de los documentos citados por el Señor Examinador considera específicamente una combinación del PNU166196 con doxorubicina o con CPT-11, por lo que la presente solicitud es novedosa frente a las enseñanzas del arte previo, tal como me permito mostrar a continuación.

5.1. Documento WO9804524 (D1).

Este documento revela compuestos derivados de acrilil distamicina, indicando de dichos compuesto son útiles como agentes antineoplásicos y antivirales. Dichos compuestos se agrupan bajo la fórmula general (I):



Me permito resaltar que dentro de los ejemplos específicos de realizaciones preferidas de la invención que son revelados en el documento D1 (páginas 4 a 7), **no se considera específicamente el PNU 166196**, y aunque los compuestos allí revelados guardan semejanza estructural con dicho PNU 166196, en el documento D1 no se consideran específicamente las sustituciones sobre la estructura central que condujeran a dicho compuesto.

De igual forma, el documento D1 hace referencia a que los compuestos de la invención pueden estar acompañados por "un agente antitumoral adicional" (página

29, líneas 15 a 19, D1), enumerando una lista de posibles compuestos que son tradicionalmente empleados como agentes antitumorales. Sin embargo, dicho documento D1 no revela de forma puntual la combinación de un derivado de acrilóil distamicina **únicamente** con doxorubicina o CPT-11, como sí lo hace el producto de la invención.

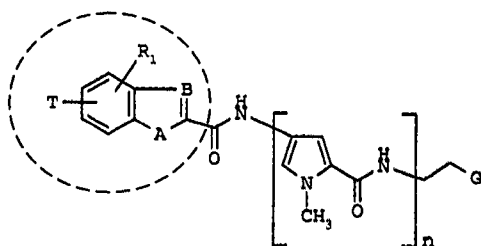
Como se ha expuesto anteriormente, dicha combinación del PNU 166196 con un doxorubicina o CPT-11, presentan un efecto sorprendentemente superior en el tratamiento antineoplásico, comparado con el uso de los componentes por separado.

De esta forma, si se tiene en consideración que el documento D1 no revela específicamente un producto que comprenda el PNU 166196 junto con doxorubicina ó CPT-11, es claro que dicho documento no anticipa de manera alguna la presente solicitud.

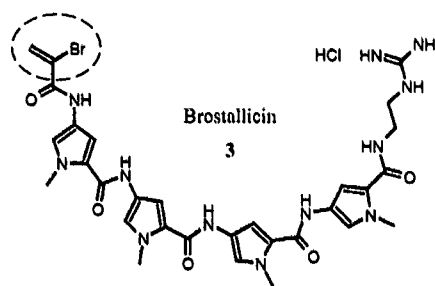
5.2 **Documento WO9950266 (D2).**

Por otro lado, el documento D2 muestra derivados benzoheterocíclicos de distamicina, y revela su uso como agentes antitumorales.

Como se expuso anteriormente, las modificaciones realizadas al capítulo reivindicatorio establecieron que el derivado de distamicina presente en los productos de la invención es el PNU 166196; si se comparan las estructuras de los compuestos revelados en D2 con el derivado de distamicina preferido de la invención:



Formula (I), D2



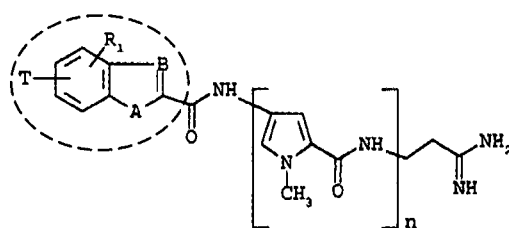
PNU 166196

Se observa claramente que el grupo benzoheterocíclico contenido en todos los compuestos revelados en D2 (señalado en línea punteada en la figura), no está presente en el compuesto preferido de la invención, por lo que a partir de dicha fórmula general (I) no sería posible construir el PNU 166196.

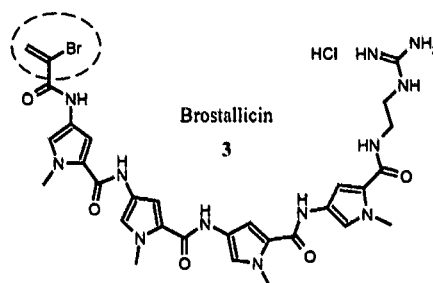
Considerando este hecho, sería evidente que el documento D2 no puede anticipar los productos de la invención.

5.3 Documento WO9821202 (D3).

Al igual que en el caso anterior, el documento D3 revela derivados benzoheterocíclicos de distamicina como agentes antineoplásicos. Al comparar la fórmula (I) del documento D3 con la estructura del PNU 166196:



Formula (I), D3



PNU 166196

Es evidente que dicho PNU 166196, no contiene el anillo de benzoheterocíclico que existe en todos los compuestos enseñados en D3, por lo que a partir de la fórmula (I) de dicho documento no sería posible construir el derivado de distamicina presente en los productos de la invención.

Consecuentemente, el documento D3 no podría anticipar los productos de la invención.

En este punto me permito referirme a lo que señala el Manual Andino para el examen de patentes con respecto a la novedad de una solicitud:

*"Si todas las características técnicas de la reivindicación independiente se encuentran descritas en un mismo antecedente y además se encuentran íntimamente relacionadas, el objeto de dicha reivindicación carece de novedad. Si una característica, aunque sea banal, no se encuentra contenida en el antecedente, la reivindicación es nueva." **

* Secretaria General de la Comunidad Andina "Manual para el examen de solicitudes de patentes de invención en las oficinas de propiedad industrial de los países de la Comunidad Andina". Lima, 2004. Página 66.

Así, si se tiene en consideración el hecho que ninguno de los documentos citados contiene de forma explícita un producto con la combinación de N-(5-{{(5-{{(5-{{(2-{{[amino(imino)metil]amino} etil) amino]carbonil}-1-metil-1H-pirrol-3-il)amino]carbonil}-1-metil-1H-pirrol-3-il)amino]carbonil}-1-metil-1H-pirrol-3-il)-4-[(2-bromoacriloil)amino]-1-metil-1H-pirrol-2-carboxamida, PNU 166196, con doxorubicina ó CPT-11, es claro que existen diferencias significativas entre la solicitud en estudio y dichos documentos, por lo que la presente solicitud es **novedosa** frente al estado del arte más próximo.

6. **CONCLUSIÓN.**

Para finalizar, me permito manifestar que teniendo en consideración el nuevo capítulo reivindicatorio, la presente solicitud es clara al describir y reclamar los productos de la invención.

De igual forma, dicho nuevo capítulo reivindicatorio no incluye reivindicaciones relativas a usos.

Sumado a lo anterior, los argumentos discutidos anteriormente establecieron de forma incontrovertible que los productos de la invención no están incluidos de manera alguna en los documentos citados, siendo por tanto la presente solicitud **novedosa** frente al estado del arte más próximo.

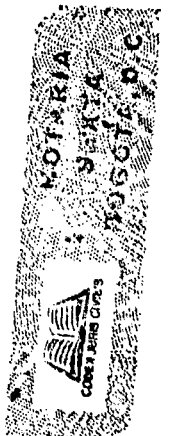
7. Anexo me permito presentar:

- (a) Recibo número 08-48,365, expedido por la Superintendencia de Industria y Comercio, que acredita el pago de las modificaciones mencionadas;
- (b) Copia del nuevo Capítulo Reivindicatorio, **que reemplaza al anterior**, en el cual se han realizado las modificaciones expuestas anteriormente; y
- (c) Copia del documento *Clinical Cancer Research*, **2003**, 9, 5402-5408, referenciado en el texto.

8. En vista de todo lo anterior, ruego a usted proceder a estudiar nuevamente la solicitud a la luz de las argumentaciones presentadas y de las modificaciones realizadas y conceder el privilegio de patente solicitado.

Señor Superintendente,


ALICIA LLOREDA RICAURTE
T.P. 53.215




ALICIA LLOREDA RICAURTE

C.C. No. 39.690.713 de Usaquén



SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

NIT : 800176089-2

RECIBO OFICIAL DE CAJA : 08 - 48,365
FECHA : JUNIO 17 DE 2008

***** CONSIGNACION *****

DEPOSITANTE	TIPO PAGO	BANCO	CUENTA	No. PAGO	FECHA PAGO	Vr. PAGO
LA Y CIA S. A	CONSIGNACION	BANCO DE BOGOTA	062754387	2756081	17/06/2008	1,182,200.00

***** CONCEPTO *****

CANT.	RENTISTICO	CONCEPTO	TOTAL CONCEPTO
1	50005-01-03	CAMBIO DE MODALIDAD Y MODIFICA	
		12 MARCAS Y LEMAS COMERCIALES	103,000.00
		TOTAL :	\$ 103,000.00

SON: CIENTO TRES MIL PESOS

RESPONSABLE :

BO DE CAJA APLICADO AL EXPEDIENTE No. _____

REIVINDICACIONES

1. Productos que comprenden clorhidrato de N-(5-{[(5-
^u ⁶
 {[(5-{[(2-{[amino/imino/metil]amino} etil) amino]carbonil}-
^Δ ^Δ
 1-metil-1H-pirrol-3-il) amino]carbonil}-1-metil-1H-pirrol-3-
⁶ ^u
 il) amino]carbonil}-1-metil-1H-pirrol-3-il)-4-[(2-
 bromoacriloil) amino]-1-metil-1H-pirrol-² carboxamida y un
 inhibidor de topoisomerasa seleccionado del grupo que
 consiste de doxorubicina y CPT-11 como una preparación ^{→ Irinotecan}
 combinada para uso simultáneo, separado o secuencial en el
 tratamiento de tumores.
2. Productos de acuerdo con la reivindicación 1 donde el
 inhibidor de topoisomerasa es CPT-11.
3. Productos de acuerdo con la reivindicación 1 donde el
 inhibidor de topoisomerasa es doxorubicina.

Enhancement of *in Vivo* Antitumor Activity of Classical Anticancer Agents by Combination with the New, Glutathione-Interacting DNA Minor Groove-Binder, Brostallicin

Maria Antonietta Sabatino, Tina Colombo, Cristina Geroni, Sergio Marchini, and Massimo Broggin¹

Laboratory of Molecular Pharmacology, Istituto di Ricerche Farmacologiche "Mario Negri," Milan [M. A. S., T. C., S. M., M. B.], and Pharmacia Italy, Discovery Research Oncology, Nerviano (Milan) [C. G.], Italy

ABSTRACT

Purpose: Brostallicin (PNU-166196) is a α -bromoacrylic DNA minor groove binder, currently in clinical evaluation. This drug has the peculiarity of showing enhanced antitumor activity in cells with high glutathione *S*-transferase (GST)/glutathione content. The purpose of the study was to study multiple combinations of brostallicin with classical anticancer agents.

Experimental Design: The *cis*-dichloro-diammine-platinum (cDDP)/brostallicin combination was tested in the human colon carcinoma (HCT-116) model transplanted in nude mice. Two treatment schedules were tested: cDDP followed by brostallicin 48 h after or brostallicin followed by cDDP. These two schemes were selected from the observation that tumor cells *in vitro* show an increased activity of GST 48 h after cDDP treatment. The HCT-116 model was used also to test the irinotecan (CPT-11)/brostallicin combination. The effect of brostallicin in combination with doxorubicin (DX) was studied in the i.v. injected murine L1210 leukemia. Three administration schedules were tested. The antitumor activity of brostallicin and Taxotere was tested on the A549 lung cancer xenografts.

Results: In line with the increased GST activity observed after treatment with cDDP, the cDDP/brostallicin interaction was sequence-dependent, leading to a more than additive antitumor effect, without additional toxicity, only when cDDP was given before brostallicin. The antitumor effect of CPT-11 was enhanced significantly by brostallicin cotreatment. A more than additive antitumor effect, without additional toxicity, was observed when DX/brostallicin were

sequentially administered in L1210-bearing mice. Finally, additivity was observed when brostallicin/Taxotere simultaneous combination was tested.

Conclusions: Although the precise molecular mechanism of interaction between brostallicin and the other tested cytotoxics has not yet been identified, a clear therapeutic gain is observed in preclinical models when brostallicin is combined with anticancer agents such as cDDP, DX, CPT-11, and Taxotere. These results indicate the potential therapeutic value of brostallicin in cancer combination treatment therapy.

INTRODUCTION

Brostallicin (*N*-[5-({[5-({[2-(aminoiminomethyl)amino]ethyl)amino]carbonyl]-1-methyl-1H-pyrrol-3-yl}amino) carbonyl]-1-methyl-1H-pyrrol-3-yl]-4-[(4-[(2-bromo-1-oxo-2-propenyl)amino]-1-methyl-1H-pyrrol-2-yl)carbonyl]amino]-1-methyl-1H-pyrrole-2-carboxamide, PNU-166196) is a new derivative of a distamycin A-like frame (1-4), which has shown very promising activity in experimental tumor models (5, 6) and is currently under clinical investigation (7, 8).

In addition, brostallicin has shown a particularly favorable therapeutic index in preclinical studies, with myelotoxicity as dose-limiting toxicity (4, 6); its cytotoxic activity is not affected by mechanisms of resistance associated to mismatch repair deficiency or to treatment with alkylating agents and topoisomerase I inhibitors (4, 6, 9).

As for its mechanism of action, brostallicin is a DNA minor groove-binding agent of which the *in vitro* and *in vivo* activity is increased in tumor cells with higher GSH² and/or GST levels (4, 10, 11). The α -bromoacrylic moiety of brostallicin was found to react with GSH, in a reaction catalyzed by GST, with the possible formation of a highly reactive GSH-complex able to bind covalently to DNA. Among the different GST isoenzymes tested, the GST- π isoform was found to be the strongest activator of brostallicin-GSH reaction. Furthermore, transfection into tumor cells of the human cDNA encoding for GST- π resulted in an increased activity of the compound both in *in vitro* and *in vivo* experimental tumor models (10). This finding is particularly interesting, considering that GSTs, especially GST- π isoform, are overexpressed with high frequency in a

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² The abbreviations used are: GSH, reduced glutathione; CI, combination index; CPT-11, irinotecan; cDDP, *cis*-dichloro-diammine-platinum; DX, doxorubicin; FTV, fractional tumor volume; GST, glutathione *S*-transferase; ILS%, percent increase in life span (ILS% = [(median survival time of treated group / median survival time of control group) $\times$ 100]-100); LCK, log cell kill; NSCLC, non-small cell lung cancer; RTV, relative tumor volume; Taxotere, docetaxel; T/C%, tumor growth inhibition.

wide variety of tumors, whereas GST levels in normal or surrounding tissues are low (12–16), thus, potentially representing a therapeutic advantage for brostallicin, rather than a mechanism of resistance as it has been observed for established antitumor agents such as platinum derivatives, alkylating agents, anthracyclines, and to a certain extent for topoisomerase I inhibitors (17–20). Moreover it has been shown that anticancer drugs such as cDDP, DX, or CPT-11 are able to promote the expression or to increase the nuclear level of GST- π in tumor cells (21, 22).

The peculiar mechanism of chemical interaction of brostallicin with GSH and particularly its enhanced activity in cells with high GST/GSH content, prompted us to investigate its efficacy in combination with drugs of which the mechanism of resistance is because of high GST/GSH levels. Theoretically these combinations should increase the number of killed cancer cells.

Because the combined use of anticancer agents has generally been the most effective approach for treating metastatic cancer than the use of single agents, most current chemotherapy regimens are based on combinations.

In the current article, we report data obtained testing the antitumor efficacy of brostallicin in combination with cDDP, CPT-11, DX, and Taxotere. We show that indeed brostallicin is suitable for combination treatments with other anticancer agents and shows synergistic activity in different tumor models.

MATERIALS AND METHODS

Drugs. Brostallicin, DX, and CPT-11 were provided by Pharmacia (Milan, Italy). cDDP was purchased from Sigma Chemical Co. (St. Louis, MO), and Taxotere was obtained from Aventis Pharmaceuticals. Drugs were dissolved and diluted just before use.

Animals. Four to 6-week-old female Swiss Ncr *nu/nu* mice (Charles River, Calco, Lecco, Italy), weighing 20–25 g, were used in experiments involving human tumors. Two to 3-month-old female DBA2 and CDF1 mice (Charles River), weighing 18–20 g, were used for L1210 cells. Mice were maintained under specific pathogen-free conditions, and provided food and water *ad libitum*.

Procedures involving animals and their care are conducted in conformity with the institutional guidelines that are in compliance with national (D.L. n.116, G.U., suppl.40, 18 febbraio 1992, Circolare No.8, G.U., 14 luglio 1994) and international laws and policies (EEC Council Directive 86/609, OJ L 358,1, Dec 12, 1987; Guide for the use of Laboratory Animals, United States National Research Council, 1996).

Tumor Cell Lines. The human tumors (HCT-116 colon carcinoma and A549 NSCLC) were obtained from American Type Culture Collection (Rockville, MD) and were maintained *in vitro* as continuous cultures. HCT-116 and A549 cells were grown in Iscove's medium or Ham's F-12, respectively, at 37°C, 5% CO₂ in medium supplemented with 10% FCS.

For drug testing, tumor cells were implanted s.c. into the left flank of recipient athymic mice (5×10^5 cells/mouse). When the tumor was palpable (~0.2 g), animals were divided randomly into test groups consisting of at least 8 mice each (day 0). L1210 murine leukemia (National Cancer Institute, Frederick, MD) was maintained by weekly i.p. transplants (10^5

cells/mouse) in DBA/2 mice, according to Geran *et al.* (23). For combination experiments, CD2F1 mice were injected i.v. with 10^6 cells/mouse. Treatments started the day after tumor inoculum.

Drug Administration and Testing. All of the drugs were administered i.v. in a volume of 10 ml/kg of body weight, according to the indicated schedules. For solid tumors, the length (L) and width (W) of the solid tumor mass were measured by caliper twice weekly, and the tumor volume (TV) was calculated as: $TV = (L \times W^2)/2$.

The tumor volume at day n was expressed as RTV according to the following formula $RTV = TV_n/TV_0$, where TV_n is the tumor volume at day n and TV_0 is the tumor volume at day 0.

The percentage of T/C% was determined by calculating RTV as: $T/C\% = 100 \times (\text{mean RTV of treated group}) / (\text{mean RTV of control group})$. According to the National Cancer Institute standards, a T/C <42% is the minimum level for activity. The LCK was calculated from the following formula: $LCK = T-C/3.32 \times Td$; T-C, the tumor growth delay, was calculated as the median time (in days) required for the treated-group tumors less the median time required for the control-group tumors to reach a predetermined size (usually 1 g), whereas Td is the tumor volume doubling time, in days, measured from a best-fit straight line of the control group in exponential growth. The compound was considered active when the LCK value was >0.7.

Drug activity for leukemia models was calculated as ILS% of treated animals compared with the control group.

Toxicity was evaluated on the basis of weight loss. Mice body weight was recorded the same day of tumor measurement and was taken as a measure of toxicity, together with gross autopsy findings, mainly in terms of reduction of spleen and liver size. Antitumor activity was evaluated at the highest non-toxic dose, which is the highest dose that can be administered without causing death or undue toxicity. A dose producing a weight loss nadir of $\geq 20\%$ was considered as excessively toxic.

Statistical Analysis. Tumor volumes of all of the groups were compared using Fisher's test.

Analyses were computed using the StatView statistical package by SAS Institute Inc. (Third edition).

The antitumor activity of the combination therapy of brostallicin plus cDDP, CPT-11, and Taxotere in solid tumor-bearing mice was analyzed by the fractional product method as described (24, 25).

FTV relative to controls is calculated as the ratio between the mean tumor volume of the experimental groups and the mean tumor volume of the control group. The calculation was performed during the time after the last treatment. The expected FTV of the combination is the product of the mean FTV of the two drugs given as single drugs. The ratio was obtained by dividing the expected FTV by the observed FTV of the combination. A ratio of >1 indicates a more than additive effect, and a ratio of <1 indicates a less than additive effect (24, 25). The antitumor activity of the combination therapy in L1210-bearing mice of brostallicin plus DX was evaluated as reported by Izumi *et al.* (26).

Measurement of GST Activity. Exponentially growing HCT-116 cells were seeded in T-25 flasks 48 h before treatment. Cells were exposed to cDDP (12, 25, and 50 μM) for 1 h and

Table 1 Combination effects of brostallicin plus cDDP against HCT-116 human colon carcinoma xenograft (sequential treatment)
Tumors were implanted s.c. in nude mice; when tumors reached 0.2 g, animals were divided into test groups (day 0). Drug was administered i.v. from day 0.

Treatment schedule ^a	Dose (mg/kg/injection)		T/C (%) ^b	FTV ^c	LCK ^d	T-C (days) ^e	Toxic deaths	Weight loss % (days of nadir) ^f
	cDDP	Brostallicin						
(A)	1	0	99	—	0	0	0/8	<5 (4)
	2	0	88	—	0.3	5	1/16	<5–13 (1)
	4	0	76	—	0.3	5	1/8	26 (1)
	0	0.2	73	—	0.2	3	0/8	8 (4)
	0	0.4	58 ^g	—	0.6	11	0/16	12 (4)
	0	0.8	53	—	1.2	23	2/8	15 (6)
	1	0.2	71	1	0.2	4	0/8	<5 (4)
	1	0.4	48 ^h	1	0.6	11	0/8	6 (4)
	2	0.2	46	1.2	0.8	14	0/8	<5 (4)
	2	0.4	40 ^{g,h,i}	2.6	1.5	27	0/16	12 (4)
	2	0.8	40 ^h	1.5	1.6	31	0/8	13 (6)
	4	0.4	52 ^j	1.1	0.9	18	2/8	22 (4)
	4	0.8	32 ^h	1.4	1.6	31	6/8	30 (4)
(B)	2	0	96	—	0.2	4	0/16	5 (7)
	4	0	78	—	0.4	10	1/16	4–13 (7)
	0	0.4	43	—	0.6	14	0/8	<5 (6)
	0	0.8	31	—	0.4	13	2/8	9 (8)
	2	0.4	61	0.7	0.4	10	0/8	9 (6)
	2	0.8	45	0.9	0.4	12	1/8	9 (8)
	4	0.4	39	0.8	0.8	19	1/8	19 (6)
	4	0.8	25 ^h	1.0	0.6	19	3/8	9 (1)

^a Administration schedules were: A, cDDP on day 0, 7, 14 and brostallicin on day 2, 9, 16; B, brostallicin on day 0, 7, 14, and cDDP on day 2, 9, 16.

^b Tumor regression (T/C%) on day 7 after the last treatment.

^c Fractional tumor volume relative to controls was calculated as described in "Materials and Methods."

^d Log cell kill.

^e T-C, tumor growth delay.

^f Days after the last treatment.

^g One of 8 mice tumor-free survivor.

^h $P < 0.01$ versus cDDP alone.

ⁱ $P < 0.01$ versus brostallicin alone.

^j $P < 0.05$ versus cDDP alone.

then incubated in drug-free medium for 24 and 48 h. Treated and untreated cells were harvested. Total GST activity was determined using 1-chloro-2-4-dinitrobenzene as a substrate (27). Reaction was performed using cytosolic extracts and measuring the conversion of 1-chloro-2-4-dinitrobenzene by GST using a spectrophotometer. The data were normalized for the amount of proteins present in each sample and expressed as nmol of dinitrophenylglutathione formed/min/mg protein at 37°C, using the extinction coefficient of $9.6 \text{ nm}^{-1} \text{ cm}^{-1}$.

RESULTS

The antitumor activity of brostallicin in combination with classical anticancer agents acting with different mechanisms has been evaluated *in vivo* in different experimental systems.

The MLH1-deficient human colon carcinoma cell line HCT-116, transplanted s.c. in nude mice, was used to determine the effect of brostallicin given alone or in combination with the platinum derivative cDDP (Table 1; Fig. 1). Mice were treated when tumors reached ~200 mg. Brostallicin and cDDP were given i.v. three times every 7 days (q7dx3). Two treatment schedules were tested: cDDP followed by brostallicin 2 days after (schedule A) or brostallicin followed by cDDP 2 days after (schedule B).

The rationale in selecting these two administration schedules was based on the observation that tumor cells treated with cDDP showed an increased activity of GST.

In fact, when GST activity was analyzed in HCT-116 cells, 24 and 48 h after a 1 h exposure to cDDP (Fig. 2), a statistical significant increase of GST activity was observed after 1-h treatment with cDDP (25–50 μM) and 48-h recovery in drug-free medium.

In all of the experiments performed (Table 1), drugs alone produced a dose-dependent inhibition of tumor growth. The highest nontoxic doses were 2 and 0.4 mg/kg/day for cDDP and brostallicin, respectively. When these two doses were combined, no addition of toxicity was found for both schedules.

As far as the antitumor activity is concerned, the effect obtained with the schedule A was significantly different from the extent of growth inhibition observed after the inverted administration schedule. The analysis of the results obtained with these two combination schedules are reported in Table 1. When cDDP administration was followed by brostallicin 48 h after, the ratio between the expected FTV and the observed FTV of the combination ranged from 1.0 to 2.6, which is clearly indicative of an increased activity of the combination over

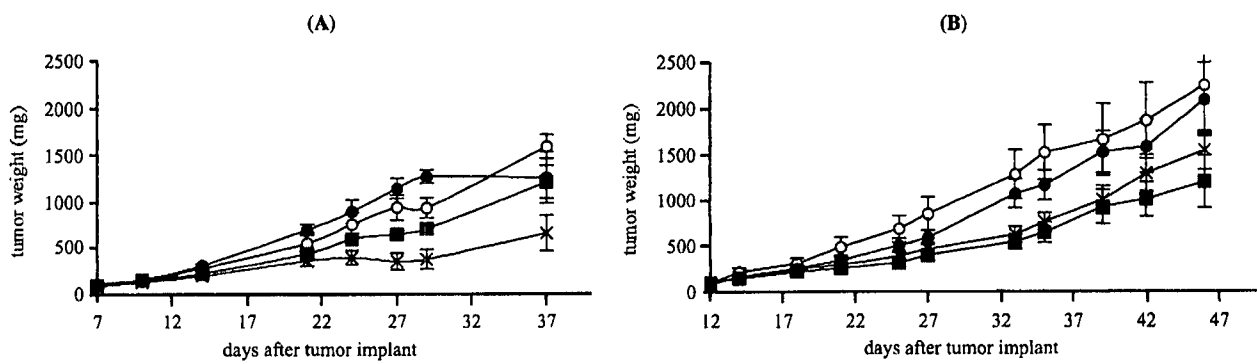


Fig. 1 Representative growth inhibition curves of brostallicin and cDDP alone and in combination in HCT-116 tumor. Mice were treated i.v. q7dx3 with brostallicin 0.4 mg/kg/injection (■) and cDDP 2 mg/kg/injection (●) alone or in combination (X) as cDDP followed by brostallicin 2 days after (A) or brostallicin followed by cDDP 2 days after (B). Control mice were treated with saline (O). Tumor volumes were measured as described in "Materials and Methods" and the mean tumor weight was plotted against time (days); bars, $\pm$ SE.

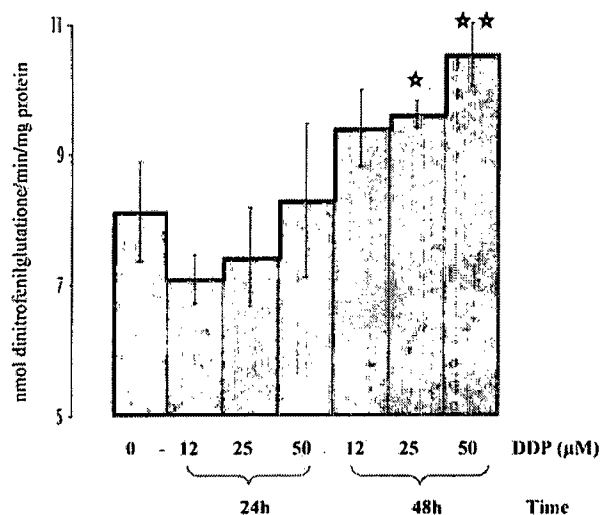


Fig. 2 Increase of GST activity in HCT-116 cells treated with cDDP. Exponentially growing cells were exposed to cDDP or to cDDP vehicle (control) for 1 h. After incubation for 24 and 48 h in drug-free medium at 37°C the level of GST activity was determined. $\star$, $P < 0.05$; $\star\star$, $P < 0.01$ versus untreated cells.

single drugs. Conversely, with the opposite schedule, the ratio ranged from 0.7 to 1, suggesting at best an additive effect of the combination.

Furthermore, schedule A was associated with a significant LCK increase (see Table 1) compared with both cDDP and brostallicin given alone.

On the contrary, LCK did not change significantly in schedule B in comparison to either drug alone.

The same tumor model was used to evaluate the efficacy of brostallicin in combination with the topoisomerase I inhibitor CPT-11. Drugs were given on the same day (q7dx3), with brostallicin administered 1 h after the i.v. injection of CPT-11. The results obtained are shown in Table 2. The highest dosages evaluated, 0.4 and 60 mg/kg/injection for brostallicin and CPT-11, respectively, were well tolerated with weight loss <5%

either when the drugs were given alone or in combination. The combination therapy exhibited more potent antitumor activity than single-drug treatment. The LCK values for CPT-11 40 mg/kg, brostallicin 0.2 mg/kg, and the combination of the two were in fact 0.4, 0.1, and 1.1, respectively. Higher doses, still not toxic, such as CPT-11 60 mg/kg, brostallicin 0.4 mg/kg, and the combination of these two, gave a LCK of 0.8, 0.4, and 1.7, respectively. The combined use of brostallicin and CPT-11 appeared to be superior to single drugs against this tumor model, being the ratio between expected and observed FTV >1 for all of the tested doses (Table 2).

The antileukemic effect of brostallicin in combination with the anthracycline DX alone was studied in the i.v. injected murine L1210 leukemia. Three administration schedules were tested alone and in combination: simultaneous administration at day 1 after tumor inoculum (schedule 1) or at 24 h interval, with DX given at day 1 and brostallicin at day 2 (schedule 2) or *vice versa* (schedule 3).

Table 3 reports the data obtained testing brostallicin, DX, and the combination of the two drugs. At all of the tested doses the CI values were determined (Table 3). The antileukemic activity of brostallicin and DX administered alone was dose dependent. The highest nontoxic doses were 0.8 and 15 mg/kg/injection for brostallicin and DX, respectively. The combination therapy of the two drugs showed a higher ILS% compared with either drug alone, especially in the DX or brostallicin-preceding treatments (schedules 2 and 3). The CI values for the three schedules ranges between 0.67 and 1, 1 and 1.52, and 1.1 and 1.65 for schedules 1, 2, and 3, respectively. These results suggest that the sequential administration of brostallicin and DX gave an antitumor effect higher than that obtained with simultaneous therapy. In all of the treatment schedules both drugs were well tolerated.

A comparison between the antitumor activity of brostallicin and Taxotere alone and in combination against A549 NSCLC transplanted in nude mice is reported in Table 4. Drugs were given simultaneously (q7dx3), with brostallicin administered 1 h after the i.v. injection of Taxotere. The highest dosages evaluated, 0.4 and 20 mg/kg/injection for brostallicin and Taxotere, respectively, were well tolerated when drugs were admin-

Table 2 Combination effects of brostallicin plus CPT-11 against HCT-116 human colon carcinoma xenograft (simultaneous treatment)

Tumors were implanted s.c. in nude mice; when tumors reached 0.2 g, animals were divided into test groups (day 0). Drug was administered i.v. from day 0; simultaneous administration once a week $\times$ 3 injections. Brostallicin administered 1 h after CPT-11.

Dose (mg/kg/injection)		T/C (%) ^a	FTV ^b	LCK ^c	T-C (days) ^d	Toxic death	Weight loss %
CPT-11	Brostallicin						
0	0.2	80	—	0.1	2	0/8	<5
0	0.4	68	—	0.4	11	0/8	<5
40	0	46	—	0.4	11	0/8	<5
60	0	39	—	0.8	23	0/8	<5
40	0.2	28 ^{e,f}	1.8	1.1	33	0/8	<5
40	0.4	32 ^{e,f}	1.1	0.9	26	0/8	<5
60	0.2	29 ^f	1.4	1	31	0/8	<5
60	0.4	19 ^{e,f}	1.2	1.7	51	0/8	<5

^a Tumor regression (T/C%) on day 7 after the last treatment.

^b Fractional tumor volume relative to controls was calculated as described in "Materials and Methods."

^c Log cell kill.

^d T-C, tumor growth delay.

^e $P < 0.05$ versus CPT-11 alone.

^f $P < 0.01$ versus brostallicin alone.

Table 3 Combination effect of brostallicin plus doxorubicin against disseminated L1210 murine leukemia

L1210 cells (1×10^5) were inoculated i.v. to CDF1 mice on day 0 and treatment was given i.v.

Dose (mg/kg/day)		Schedule 1 DX (day 1) Brostallicin (day 1)			Schedule 2 DX (day 1) Brostallicin (day 2)			Schedule 3 Brostallicin (day 1) DX (day 2)		
Brostallicin	DX	ILS% ^a	Tox ^b	CI ^c	ILS% ^a	Tox ^b	CI ^c	ILS% ^a	Tox ^b	CI ^c
0.2	0	38	0/8	—	8	0/8	—	14	0/8	—
0.4	0	23	0/8	—	17	0/8	—	14	0/8	—
0.8	0	69	0/8	—	33	0/8	—	50	0/8	—
0	7.5	54	0/8	—	33	0/8	—	29	0/8	—
0	15	100	0/8	—	67	0/8	—	57	0/8	—
0.2	7.5	69	0/8	0.75	42	0/8	1.02	57	0/8	1.33
0.2	15	92	0/8	0.67	100	0/8	1.33	86	0/8	1.21
0.4	7.5	78	0/8	1.01	58	0/8	1.16	71	0/8	1.65
0.4	15	108	0/6	0.88	100	0/8	1.19	93	0/8	1.31
0.8	7.5	100	0/8	0.81	100	0/8	1.52	86	0/8	1.1
0.8	15	123	0/8	0.73	100	0/8	1	114	0/8	1.1

^a Percentage increase in life span.

^b Number of toxic deaths/number of mice.

^c Combination index = ratio between the ILS% of the two drugs in combination and the sum of ILS% of brostallicin and DX alone. A CI of >1 indicates a more than additive effect, and a ratio of <1 indicates a less than additive effect.

istered alone. Conversely, when drugs were given in combination at these dosages, some initial toxicity could be observed. Against this tumor model, Taxotere was very active (LCK of 8.3 at 20 mg/kg/injection), and brostallicin did not show any significant antitumor activity. When combination therapy was performed with the highest tolerated doses of both drugs, the antitumor effect was additive.

DISCUSSION

Brostallicin is a novel DNA minor groove binder anticancer agent in clinical development. The combined use of anticancer agents is a common strategy in clinical chemotherapy and, thus, the possibility to identify optimal combinations of the antitumor agents should be examined. The results of our study suggest that brostallicin is not only an active compound in single-agent therapy but is also a promising agent in combination with other anticancer agents.

Preclinical findings showing that brostallicin has increased activity in tumors with higher GSH/GST levels/expression (4, 10, 11), overcomes resistance to alkylating agents and topoisomerase I inhibitors, and is fully effective against mismatch repair-deficient tumor cells (23), stimulated our interest in studying the combined activity of brostallicin with drugs of which the antitumor activity is reduced by these mechanisms of resistance (13–20). The involvement of GSTs in tumor resistance to a wide range of chemotherapeutic agents has been described extensively (13–20). In addition, it has been shown that GST- π expression or amount into the nucleus increases in tumor cells in response to cDDP, DX, and CPT-11 treatment (21, 22).

The interaction and the biochemical mechanism of synergy between cDDP and brostallicin have been explored on a mismatch repair-deficient model (HCT-116; Ref. 28). The kinetic of GST activity in HCT-116 cells exposed to cDDP was eval-

Table 4 Combination effects of brostallicin plus taxotere against A549 human lung carcinoma xenograft (simultaneous treatment)
Tumors were implanted s.c. in nude mice; when tumors reached 0.2 g, animals were divided into test groups (day 0). Drug was administered i.v. from day 0; simultaneous administration once a week × 3 injections.

Dose (mg/kg/injection)		T/C (%) ^a	FTV ^b	LCK ^c	T-C (days) ^d	Toxic death	Weight loss % (days of nadir) ^e	Tumor free/total mice
Taxotere	Brostallicin							
2.5	0	115	—	0	0	0/8	<5	0/8
5	0	70	—	0.09	2	0/15	11 (4)	0/15
10	0	37	—	0.62	26	0/6	11 (4)	1/6
20	0	18	—	8.3	347	0/7	11 (8)	3/7
0	0.2	86	—	0.04	2	0/15	<5	1/15
0	0.4	95	—	0	0	1/15	7 (4)	0/15
2.5	0.2	73 ^f	1.4	0	0	0/8	<5	0/8
2.5	0.4	54 ^g	1.9	0.18	4	0/8	<5	0/8
5	0.2	57 ^h	1	0.14	3	0/8	<5	0/8
5	0.4	52 ^h	1.1	0.24	9	0/15	15 (4)	0/15
10	0.4	28 ^h	1.4	2.9	122	0/6	19 (4)	0/6
20	0.2	13 ^h	1	N.E. ⁱ	N.E.	0/7	19 (8)	1/7
20	0.4	9	—	N.E.	N.E.	6/7	23 (4)	0/7

^a Tumor regression (T/C%) on day 8 after the last treatment.
^b Fractional tumor volume relative to controls was calculated as described in "Materials and Methods."
^c Log cell kill.
^d T-C, tumor growth delay.
^e Days after the last treatment.
^f *P* < 0.05 versus taxotere alone.
^g *P* < 0.01 versus taxotere alone.
^h *P* < 0.05 versus brostallicin alone.
ⁱ N.E. not evaluable because of the tumor growth kinetic value.

uated and results confirmed the previously reported ability of cDDP in increasing the expression of GST- π . Because results showed a higher level of GST activity after 48-h recovery in drug-free medium, two combination protocols were investigated giving brostallicin 48 h before or after cDDP treatment. The sequence of drug administration in the combination influenced the response. cDDP/brostallicin interaction leads to a more than additive antitumor effect without additional toxicity, only giving cDDP before the brostallicin administration. These findings support our hypothesis that cDDP could enhance brostallicin activity through GST- π -stimulated overexpression. We found that the antitumor activity of CPT-11 is enhanced by brostallicin cotreatment. Although a clear understanding of the mechanism by which the antitumor activity of these two drugs benefits of their combination is not clearly understood, the combined use of brostallicin and CPT-11 is an interesting approach for colon cancer treatment considering that brostallicin overcomes camptothecin resistance and, therefore, can kill cells that can survive to CPT-11 treatment.

Furthermore, brostallicin preserves its activity in mismatch repair-deficient tumors, a condition found in human colon cancer with relatively high frequency (29–31).

In *in vivo* systemic L1210 murine leukemia model a more than additive antitumor effect was observed when DX/brostallicin were sequentially administered. The reasons for these results still remain to be determined, and other mechanisms, different from those involving the GSH/GST system, could be operative and should be investigated for designing more effective treatments regimens.

The last study reported in this article regards the combination of brostallicin and Taxotere. The choice of this agent was guided by the brostallicin-clinical plan in which NSCLC has

been selected. Among the effective drugs in clinical use against NSCLC, Taxotere as single agent or in combination therapy provides a clinical benefit over or comparable with other agents (32, 33). Preclinical data showed that brostallicin/Taxotere simultaneous combination is feasible without any antagonistic effect.

Combination chemotherapy using conventional cytotoxic agents accomplishes several important objectives not possible with single-agent treatment. Here we report four promising brostallicin combination treatments. Brostallicin plus cDDP or CPT-11 or DX showed hints of therapeutic synergy, and brostallicin plus Taxotere activity was certainly additive. Altogether these results, together with the peculiar mechanism of action of the drug, indicate brostallicin as a new promising candidate for clinical use in combination therapy.

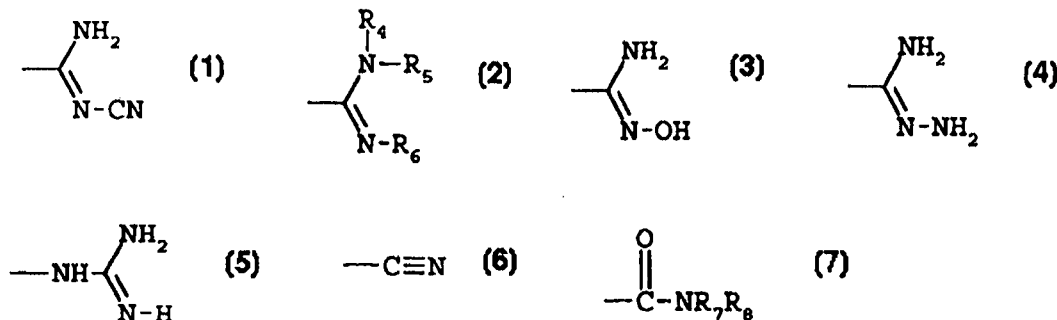
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$$\begin{array}{c} R_1 \\ | \\ R_2 - C = C - R_3 \\ | \\ R_2 \end{array} \begin{array}{c} \text{NH} \\ | \\ \text{C} = \text{O} \end{array} \left[\begin{array}{c} \text{C}_5\text{H}_4\text{N} \\ | \\ \text{CH}_3 \end{array} \right]_n \begin{array}{c} \text{NH} \\ | \\ \text{C} = \text{O} \end{array} \text{CH}_2\text{CH}_2\text{B} \quad (I)$$


Acryloyl substituted distamycin derivatives of formula (I) wherein n is 2, 3, or 4; R₁ and R₂ are selected, each independently, from: hydrogen, halogen, and C₁-C₄ alkyl; R₃ is hydrogen or halogen; B is selected from: (1), (2), (3), (4), (5), (6) and (7); wherein R₄, R₅, R₆, R₇, and R₈ are, each independently, hydrogen or C₁-C₄ alkyl, with the proviso that at least one of R₄, R₅ and R₆ is C₁-C₄ alkyl; and pharmaceutically acceptable salts thereof. Such compounds are useful as antineoplastic and antiviral agents.

animal body by therapy.

Furthermore, the present invention provides a method for treating tumors and viral infections in a patient in need of it, which comprises administering to said patient a
5 composition of the invention.

A further object of the present invention is a combined method for treating cancer or for ameliorating the conditions of mammals, including humans, suffering from cancer, said method comprising administering a compound of
10 formula (I), or a pharmaceutically acceptable salt thereof, and an additional antitumor agent, close enough in time and in amounts sufficient to produce a therapeutically useful effect.

15 The present invention also provides combined preparations for simultaneous, separate or sequential use in anti-cancer therapy, comprising a compound of formula (I), or a pharmaceutically acceptable salt thereof, and an additional antitumour agent.

20 The term "antitumor agent" is meant to comprise both a single antitumor drug and "cocktails" i.e. a mixture of such drugs, according to the clinical practice. Examples of antitumor agents that can be formulated with a compound of formula (I), or alternatively, can be administered in a
25 combined method of treatment, include [doxorubicin], daunomycin, epirubicin, idarubicin, etoposide, fluoro-uracil, melphalan, cyclo-phosphamide, 4-demethoxy daunorubicin, bleomycin, vinblastin, and mitomycin, or mixtures thereof.

30

The following examples are given to better illustrate the invention, but do not limit the scope of the invention

FAB-MS: m/z 724, (10, [M+H]⁺)

PMR (DMSO-d₆) δ :

10.27 (s, 1H), 9.94 (s, 1H), 9.91 (s, 1H), 9.88 (s, 1H),
 9.5 (b.s., 2H), 8.5 (b.s., 1H), 7.98 (t, J=5.9 Hz, 1H),
 5 7.3-6.8 (m, 8H), 6.66 (d, J=2.9 Hz, 1H), 6.21 (d, J=2.9 Hz,
 1H), 3.84 (s, 6H), 3.83 (s, 3H), 3.79 (s, 3H), 3.38 (b.s.,
 2H), 2.31 (b.s., 2H).

UV: c=4.16mg/l (MeOH) λ_{MAX}=307.8 ε=51155

10 By analogous procedure and by using the opportune starting materials the following products can be obtained:

3-[1-methyl-4[1-methyl-4[1-methyl-4(α-bromoacrylamido)pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidoxime; and

15 3-[1-methyl-4[1-methyl-4[1-methyl-4[1-methyl-4(α-chloroacrylamido)pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidoxime.

20 EXAMPLE 5

2-[1-methyl-4⁺[1-methyl-4⁺[1-methyl-4⁺[1-methyl-4⁺(α-bromoacrylamido)pyrrole-2-carboxamido⁺]pyrrole-2-carboxamido⁺]pyrrole-2-carboxamido⁺]pyrrole-2-carboxamido⁺]pyrrole-2-carboxamido⁺]ethylguanidine hydrochloride

25

Step I The intermediate 2-aminoethylguanidine dihydrochloride

A solution of commercial N-BOC-ethylendiamine (1 g) in dry ethanol (100 ml) and 2-methyl-2-thiopseudourea hydroiodide
 30 (1.5 g) was refluxed for 8 hours. The solvent was removed at reduced pressure and the crude residue purified by flash

3-[1-methyl-4[1-methyl-4[1-methyl-4-aminopyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionitrile hydrochloride.

5 Step III The title compound

A solution of 250 mg of 1-methyl-4-(α -bromoacrylamido)pyrrole-2-carboxyl chloride (prepared as reported in Example 1 step II) in 15 ml of benzene, was added to a solution of the intermediate obtained from step II (250 mg) and 82 mg of NaHCO₃ in 5 ml of H₂O. The solution was vigorously stirred for 8 hours at room temperature, then evaporated in vacuo and the crude residue was purified by flash chromatography (methylene chloride/ methanol: 8/2) to yield 220 mg of the title compound as a yellow solid.

15 FAB-MS: m/z 723, (32, [M+H]⁺)

PMR (DMSO-d₆) δ :

10.30 (s, 1H), 9.95 (s, 1H), 9.92 (s, 1H), 9.90 (s, 1H), 8.10 (t, J=5.9Hz, 1H), 7.56 (t, J=5.9, 1H), 7.2 (b.s., 4H), 6.9-7.3 (m, 8H), 6.68 (d, J=2.9 Hz, 1H), 6.21 (d, J=2.9 Hz, 1H), 3.85 (s, 3H), 3.84 (s, 3H), 3.83 (s, 3H), 3.80 (s, 3H), 3.30 (b.s., 4H).

UV: c=15.3mg/l (EtOH95%) λ_{max} =312.0 ϵ =48792

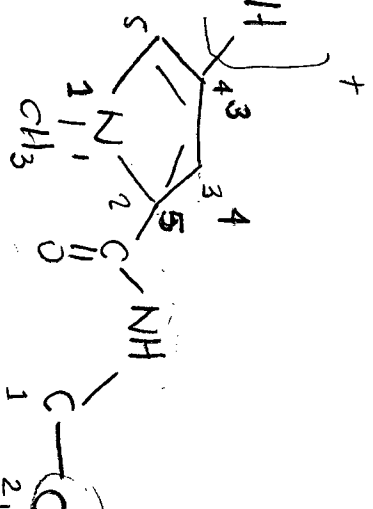
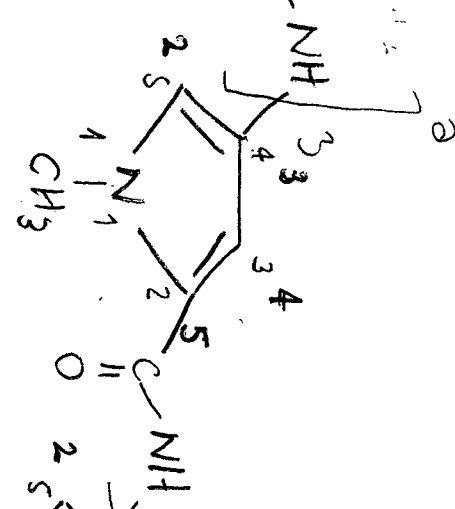
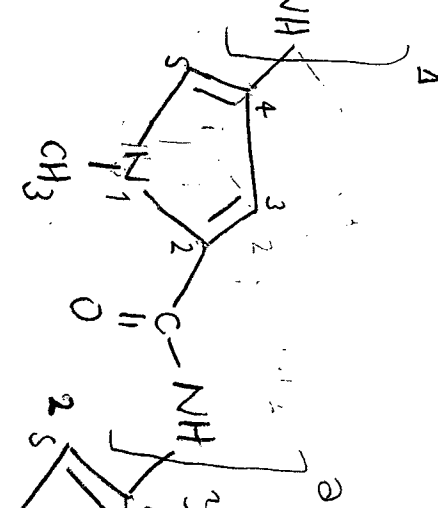
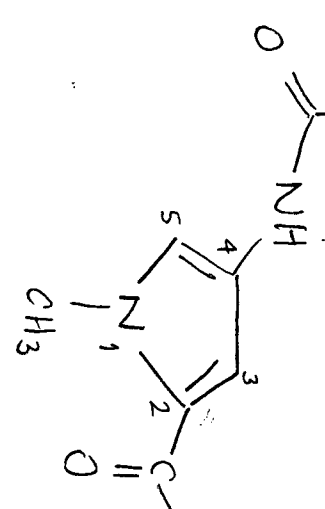
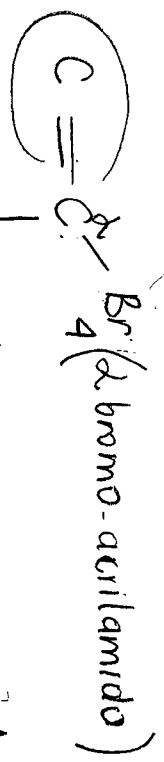
By analogous procedure and by using the opportune starting materials the following products can be obtained:

(2)-[1-methyl-4[1-methyl-4[1-methyl-4(α -bromoacrylamido)pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]ethylguanidine hydrochloride;

(2)-[1-methyl-4[1-methyl-4[1-methyl-4[1-methyl-4(α -chloroacrylamido)pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]ethylguanidine

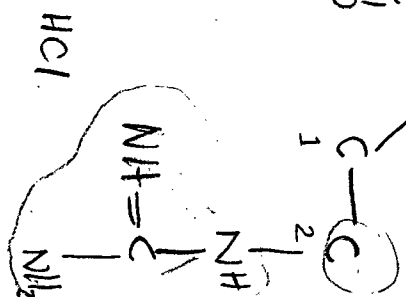
4 pyrrole

Δ



Anterioridad D_1
Nomenclatura

Solicitud
Nomenclatura



HCl

14.5



PubChem » Compound Summary

PubChem Compound
PubMed | Entrez | Structure

Brostallicin hydrochloride - Compound Summary (CID: 6918407)

alkylates DNA in the presence of cellular thiols;
structure in first source

Table of Contents

- Drug and Chemical Info
- Synonyms
- Properties
- Descriptors
- Compound Info
- Substance Info
 - Category
- Exports



Drug and Chemical Info from MeSH: (Total:1)

2

brostallicin**PubMed****PubMed MeSH Keyword Summary** 2

Depositor-Supplied Synonyms: (Total: 4)

Sort: Weight

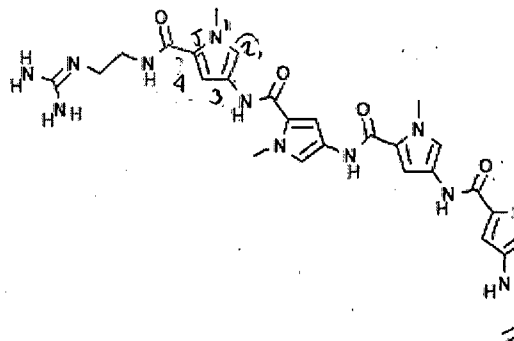
Brostallicin hydrochloride
PNU-166196A
CID6918407
PNU-166196



Properties Computed from Structure: 2

Molecular Weight	760.04124 [g/mol]
Molecular Formula	C ₃₀ H ₃₆ BrClN ₁₂ O ₅

Cl H



Compound ID	6918407
Molecular Weight	760.04124 [g/mol]
Molecular Formula	C ₃₀ H ₃₆ BrClN ₁₂ O ₅
H-Bond Donor	8
H-Bond Acceptor	12

Links

Chemical Structure Search 2

146

H-Bond Donor	8
H-Bond Acceptor	12
Rotatable Bond Count	12
Tautomer Count	64
Exact Mass	758.180353
MonoIsotopic Mass	758.180353
Topological Polar Surface Area	230
Heavy Atom Count	49
Formal Charge	0
Complexity	1280
Isotope Atom Count	0
Defined Atom StereoCenter Count	0
Undefined Atom StereoCenter Count	0
Defined Bond StereoCenter Count	0
Undefined Bond StereoCenter Count	0
Covalently-Bonded Unit Count	2



Descriptors Computed from Structure:



IUPAC Name: 4-[[4-[[4-(2-bromoprop-2-enoylamino)-1-methylpyrrole-2-carbonyl]amino]-1-methylpyrrole-2-carbonyl]amino]-N-[5-[2-(diaminomethylideneamino)ethylcarbamoyl]-1-methylpyrrol-3-yl]-1-methylpyrrole-2-carboxamide hydrochloride

Canonical SMILES: CN1C=C(C=C1C(=O)NCCN=C(N)N)NC(=O)C2=CC(=CN2C)NC(=O)C3=CC(=CN3C)NC(=O)C4=CC(=CN4C)NC(=O)C(=C)Br.Cl

InChI: InChI=1/C30H35BrN12O5.ClH/c1-16(31)25(44)36-17-9-22(41(3)12-17)27(46)38-19-11-24(43(5)14-19)29(48)39-20-10-23(42(4)15-20)28(47)37-18-8-21(40(2)13-18)26(45)34-6-7-35-30(32)33;/h8-15H,1,6-7H2,2-5H3,(H,34,45)(H,36,44)(H,37,47)(H,38,46)(H,39,48)(H4,32,33,35);1H/f/h34,36-39H,32-33H2;



Compound Info:



CID: 6918407

Create Date: 2006-07-28

Parent CID: 6918408

Unique Components: 2 Links

147

Similar Compounds: 287 Links  **Substance Info:** **Substances:** 4 Links **Category:** [for same structure substances] 

Journal Publishers: 2 Links

Prous Science Drugs of the Future (1)

SID: 12015208 - External ID: 262512

Thomson Pharma (1)

SID: 14791126 - External ID: 00076602

Substance Vendors: 1 Link

ChemSpider (1)

SID: 43529777 - External ID: 5293604



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Brachidura

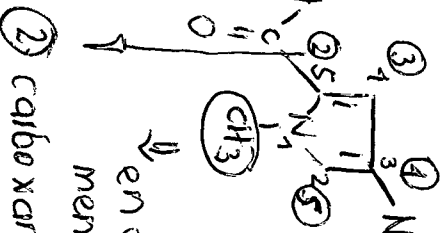
Nomenclatura reivindicada.

Br Bromo

α C=C acril

amido

N.



en esta se aplica la regla del menor número. ya no se identifica en 5 la posición sino como 2

